BRAIN DAMAGE IN DOGS RESULTING FROM PULSATILE AND NON-PULSATILE BLOOD FLOWS IN EXTRACORPOREAL CIRCULATION

A thesis submitted for the degree of Doctor of Philosophy in May, 1971 by Gordon Wright, R.M.N., B.A., M.Sc., University of Keele.

VOLUME I

Appendix 2 (published paper from 'Journal of Pathology' vol 100, no 4, pgs 295-300, 1970) has not been digitised at the request of the university.

ACKNOWLEDGEMENTS AND DECLARATION OF PARTICIPATION

This thesis contains a report of work completed in the W.E. Dunn Unit of Cardiology during the period January 1968 to December 1970. I, the author, have had the privilege of taking part in a team excercise and of learning new techniques while taking the major share of benefits to be derived from the experimental results.

Solutions to the many problems have been sought co-operatively and specialist knowledge has been obtained from many sources.

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For my part, I can claim that the light- and electron-microscopical preparations are entirely my own work. I produced the electron-micrographs and performed the cell counts. I derived the numerical values from original records and made the graphical and statistical analyses of the physiological data, cell counts and perfusion-fixation data. I performed the surgery necessary for the perfusion-fixation of control, anaesthetic and recovery animals and conducted the laboratory experiments to detect and identify circulatory emboli.

In my discussions, I have endeavoured to be original but the conclusions and hypotheses represent attempted integrations of facts and ideas derived from a variety of sources. The exchange of ideas provides an essential stimulus for experimental research work. At Keele, we feel our isolation from other medical and surgical research workers very intensely. For this reason, I am sincerely grateful for liaisons and fruitful discussions with members of the Cardiac Surgery team and Biomedical Engineering Department at the North Staffordshire Royal Infirmary; the Departments of Pathology and Physiology at the Medical School, Birmingham University; the Stafford Estate Laboratories of General Electric Company Power Engineering Limited; the Department of Haematology, Industrial Hygiene Research Laboratories; and also with my colleagues in the W. E. Dunn Unit of Cardiology. These people have filled an intellectual vacuum in which productive research would have been difficult, if not impossible.

ABSTRACT

In a review of the scientific literature, teleological, theoretical, clinical and experimental evidence is produced for expecting pulsatile flow to improve tissue perfusion compared with non-pulsatile flow. Experiments have been performed on dogs to test this hypothesis.

Histological changes occurring in the brain are compared following pulsatile and non-pulsatile whole-body perfusions. Some animals died and the possible causes of death are discussed. The physiological variables were analysed by statistical methods and significant differences between pulsatile and non-pulsatile experiments are indicated.

Early, diffuse nerve cell changes are attributed to non-pulsatile perfusion, but lower arterial carbon dioxide tensions, higher rates of haemolysis and retrograde blood flow during non-pulsatile perfusions are admitted as possible complicating factors.

Cell counts were performed with an automatic image analysing computer and variations from the control values are attributed to cell swelling and/or glial proliferation and infiltration.

Electron-microscopy of the brains of dogs perfused with non-pulsatile flow supported the histological results and provided further information about the early sub-cellular changes associated

with cell swelling and shrinkage.

Hypotheses concerning the evolution of nerve cell reactions to hypoxia and/or ischaemia, and the mechanisms by which non-pulsatile flow may result in nerve cell damage are presented.

Focal lesions, found mainly in the brains of animals that recovered for a few days post-operatively, are considered to be due to circulatory emboli of gas bubbles and aggregates of blood cells. Following in vitro and in vivo tests, some recommendations for the reduction of circulatory emboli are offered.

Several important outstanding problems remain and a programme of experiments is proposed for their clarification.

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HISTORICAL SURVEY

Introduction

The concept of extracorporeal circulation was introduced by Le Gallois in 1812, but its potential as an adjunct for open-heart surgery was not realised until the suggestion of Gibbon (1937) was implemented on a human patient by Dennis et al (1951). The period since 1955 has been noteworthy for the rapid development of new techniques of extracorporeal circulation. Pump-oxygenators are now widely used, but the technique of profound hypothermia with circulatory occlusion developed by Drew and his associates makes use of the patient's own lungs for gas exchange and gains the advantages of retention of the lungs as a filter, avoidance of blood trauma in oxygenators, and less chance of embolic damage (Drew and Anderson, 1959; Drew et al, 1959; Drew, 1961). Its disadvantages are the need for quadruple cannulation, and the necessity for a period of total circulatory arrest at low body temperature while surgical corrections are performed on the heart.

A large volume of literature records the general opinion that techniques of extracorporeal circulation currently employed for open-heart surgery cause brain damage. Reviews by Allen (1958), Clowes (1960), Galletti and Brecher (1962; 1965) and Peirce (1969) reveal that the most consistent aetiological factor in brain damage is

micro-embolism. Hypotension and reduced cerebral blood flow are thought to be responsible for brain damage in some difficult cases, but ischaemia is not implicated when normal blood pressures and flows are maintained throughout the perfusion. Only Peirce was able to draw the conclusion that it is quite likely that "pulsatility of a suitable wave form, amplitude, and frequency would produce improved perfusion ...". Previous reviewers had reached no conclusions because of unconvincing evidence. Although it had been suspected that pulsatile blood flow might be advantageous, a cautious attitude had been adopted in view of the great success of non-pulsatile perfusions.

Neurologic and Psychiatric complications of open-heart surgery

Patients presented for cardiac operations have frequently experienced low cardiac output and biochemical disturbances for several years prior to operation. They display a variety of attitudes toward surgery and have many ways of compensating for their illness. The early post-operative period can be a major trial of character, for the rapid recovery from a long illness often requires extensive revision of close relationships and a redistribution of dependencies. These pre- and post-operative factors have complicated assessments of the neurological and psychiatric effects of open-heart surgery.

Surprisingly, Patrick et al (1958) found no neurological abnormalities in 245 patients surviving open-heart surgery with a

pump-oxygenator, and Dubost et al (1962) reflecting upon their experiences with pump-oxygenators recorded no neurological accidents after either normothermic or hypothermic perfusions. Following their first seven open-heart surgery cases employing their new perfusion technique, Drew and Anderson (1959) observed no neurological symptoms in the five patients who survived cooling to 15°C.

The tests used to assess neurological defects were not described in any of these reports, so the observations must be regarded with a suspicion of subjectivity in the perspective of high incidences of neurological involvement observed by other authors.

Psychiatric complications of open-heart surgery have been reviewed up to 1965 by Hazán (1966a). The incidence of psychiatric disorders is higher after cardiac surgery than after any other major surgery, and higher after extracorporeal circulation has been employed than when it has not. Tables 1 and 2 are constructed from data derived from reports on neurological and psychiatric sequelae of cardiac surgery in which extracorporeal circulation was employed. The typical post-operative state has been called "post-cardiotomy delirium" (Blachly and Starr, 1964), or "catastrophe reaction" (Meyer et al, 1961). Clinical symptoms include hallucinations, delusions, confusion, amnesia, excitement, depression and disorientation. Symptoms become apparent within the first fourteen post-operative days and are usually temporary, the average duration being five days

(Egerton and Kay, 1964). Motor disorders are common (Silverstein et al, 1960; Ehrenhaft et al, 1961; Fowler et al 1962; Caguin and Carter, 1963; Gilman, 1965; Sachdev et al, 1967). They vary from localised muscular tremor to hemiparesis. Epileptiform seizures were observed by Ehrenhaft et al (1961), Gilman (1965) and Dobell et al (1966).

The general conclusion may be that there are two principal groups of neurological and psychiatric complications. One group is characterised by slow recovery from anaesthesia. Somnolence and effective disorders may persist for a few days and some patients die. However, the prognosis is generally good, no persistent symptoms being detectable after three months. In the second group, localised motor defects predominate but affective states, such as severe depression, are frequent. Memory loss for specific items, inability to complete simple arithmetic calculations, and loss of muscular co-ordination may also belong to this group of symptoms. Group 1 symptoms are reminiscent of generalised cerebral depression such as that which results from temporary hypoxia, while the symptoms of group 2 might be explained by localised cerebrovascular accidents such as arteriolar embolism. Local pareses and co-ordination defects may be due to involvement of the pyramidal and extrapyramidal systems, common sites for the lodgement of emboli (Blackwood, 1963).

A conclusion of this kind is compatible with the results of most neurologists and psychiatrists who have reported their

examinations of open-heart surgery patients. However another group recognises the more subtle psychological influences of impending major surgery, and post-operative stresses, particularly unusual sensory experiences in the intensive care unit, as being the critical factors (Abram 1965; Kennedy and Bakst, 1966; Morgan, 1970) or precipitating factors (Egerton and Kay, 1964; Kornfeld et al, 1965). While acknowledging the importance of psycho-pathological factors in the immediate pre- and post-operative periods, several criticisms of the above mentioned investigations may be made:

(a) Sub-classification according to neurological and psychotic reactions invalidates statistical correlations, e.g. Morgan (1970) correlated pre-operative depression with post-operative death since all four of his hopelessly depressed patients died within two weeks of surgery, but the significance of this result is reduced by the death of another eleven patients who were not hopelessly depressed in a total group of seventy-two open-heart surgery cases.

(b) There may be a good patho-physiological reason for such correlations not requiring a psychological explanation, e.g. hypoxic/ischaemic damage due to the existing cardiovascular defect.

(c) In many cases, no correlations between neurological signs and variables in the surgical procedure were sought. Exceptions to this general statement are to be found in the works of Kornfeld et al (1965), who established that the incidence of post-operative

delirium was increased by pre-operative incapacitation, but also by double-valve replacement and prolonged by-pass; Blachly and Kloster (1966), who reported a correlation between psychiatric complications and post-operative cardiac output; and of Javid et al (1969), who found advanced age, low mean arterial pressure during perfusion and prolonged by-pass to be the most significant causes of central nervous system damage during open-heart operations. Sachdev et al (1967) were unable to find any correlations between neurological and psychiatric malfunctions and anoxia, acidosis, alkalosis, electrolyte imbalance, fever, sensory deprivation, sleep deprivation, adrenal steroid dyscrasia, abnormal blood proteins and hallucinogenic agents in the blood.

(d) Microscopic examination of the brains of ten patients who died after open-heart surgery revealed diffuse, acute anoxic changes and focal infarcts in the cerebral cortex in the series of Javid et al (1969) and Ehrenhaft et al (1961) but these are the only neurological and psychiatric assessments to include histological results.

An alternative explanation offered by Hazán (1966b) is that elevated levels of circulating amines, or abnormal metabolism of these compounds could have psychotic effects. Plasma epinephrine and norepinephrine are raised during normothermic and hypothermic cardiopulmonary by-pass in human patients, and increasing serum 5-hydroxytryptamine (serotonin) increases at the same rate as

platelet destruction (Roplogle et al, 1962). Hazán's (1966b) theory is that serotonin competes with acetylcholine for receptor sites at cholinergic synapses, or that one or more of the catecholamines is abnormally metabolised to produce bufotonin-like hallucinogens. His theory is poorly substantiated by very tenuous evidence and implies a direct effect of catecholamines and serotonin upon the cerebral cortex involving only sensory fields. In fact, the highest specific activity for serotonin is found in nerve-ending subcellular fractions isolated from the hippocampus, basal ganglia and grey areas of the mesencephalon (Fiszer and De Robertis, 1969). The hypothesis of Păusescu et al (1970) is more credible. They postulate that the accumulation of catecholamines and serotonin is due to the inhibition of oxidative deamination by hypoxia. An important secondary effect is that the permeability of cell membranes is altered. Cations are redistributed by a facilitated sodium flux inward and a decreased efflux of potassium. This would seriously interfere with the depolarisation and repolarisation stages of nerve impulse transmission (Hodgkin, 1951).

Of course, this theory does not explain the initial hypoxia, but Mendler (1968) has suggested that hypoxia can be caused by cold tissue swelling, which is a sequel of increased tissue cation content in rat brain cooled to 2-4°C. The accumulation of cations is thought to be due to reduced rates of active transport in the cold brain.

Theories based upon changes in the tissue content of cations, catecholamines and serotonin appear to have some credibility, but the mechanisms involved are not understood. Cations, catecholamines and serotonin may be inter-related in complex, cyclical metabolic pathways which may be secondary to or further complicated by some other factors.

Electroencephalography

The electroencephalograph (EEG) is routinely used as a monitor of cerebral functional state during open-heart surgery. Unfortunately, it has proved to be difficult to interpret, but it provides useful information about the total level of cerebral activity during cooling and rewarming, and changes in the predominant thythm are thought to be significant.

Although Hodges et al (1958) observed no EEG abnormalities during 20-80 minute periods of cardiac surgery with a pump-oxygenator, Arfel et al (1967) used the EEG to diagnose 67 suspected cases of air embolism in a series of 1600 open-heart surgery operations. Owens et al (1958) noted EEG changes shortly after commencing by-pass with a bubble oxygenator in both patients and dogs. Fat emboli were detected in the kidneys and in brain capillaries of dogs that died within the first 24-hours, but no cell damage was found. EEG changes were observed in 2 of the 24 patients of Silverstein et al (1960), and in the experience of Sachdev et al (1967), the onset of post-operative neurological symptoms correlated with a replacement of the

normal alpha rhythm by slower theta and delta rhythms of increased amplitude. High incidences of post-operative neurological derangement and death were correlated with abnormal EEGs during cardiopulmonary by-pass by Fowler et al (1962).

Similar changes in cerebral electrical activity occur for short periods during extracorporeal circulation when the cerebral perfusion pressure is less than 45mm Hg in children (Harden, 1965), in association with a low blood oxygen saturation, at the start and completion of cardiopulmonary by-pass, when the superior vena cava is clamped, and to a lesser extent, when the inferior vena cava is clamped (Fischer-Williams and Cooper, 1964).

As a monitor of cerebral functional state, the EEG may be too insensitive to detect any but the most gross changes in cerebral perfusion, and too indiscriminate for localised lesions. A normal EEG throughout the by-pass is a poor indication of the absence of focal brain lesions which will remain undetected unless they are numerous and widely scattered.

Histopathology of human brain

With one exception (Patrick et al, 1958), neuropathological reports on the brains of patients who died during, or shortly after, open-heart surgery have always described lesions of an ischaemic type. These are classified as diffuse, or focal, or both. In cases of early death (within the first 6.5 post-operative hours) ischaemic cell change, neuronal pallor and cell loss are the only manifestations

of brain damage (Brierley, 1963). Glial reactions are evident after 48 hours. Cerebral oedema (Ehrenhaft et al, 1961; Fowler et al, 1962) or haemorrhage (Bleifield, 1961; Gilman, 1965), or both (Silverstein et al, 1960) may accompany the cell changes. The regions most likely to be affected are the parieto-occipital cortex, cerebellar Purkinje cells, basal ganglia and Ammon's horn (Brierley, 1963; 1967; Björk and Hultquist, 1960).

Diffuse cell damage is thought to be caused by anoxia (Javid et al, 1969), reduced cerebral blood flow (Brierley, 1963; 1964; 1967), hypoxaemia and carbon dioxide poisoning (Björk and Hultquist, 1960).

Focal lesions are thought to be due to cerebrovascular emboli. Air embolisation has been suggested as a cause of brain damage by Arfel et al (1967), and Fishman et al (1969) have demonstrated that air pockets formed in the pulmonary veins when the left atrium is empty may embolise several hours later. Silicone antifoam solutions used to prevent air embolism in pump-oxygenator techniques cause cerebral silicone emboli (Bleifield, 1961; Ehrenhaft et al, 1961; Lindberg et al, 1961; Thomassen et al, 1961). Pump-oxygenator techniques are also liable to produce fat emboli particularly when the pericardial blood is returned to the patient via the cardiac sucker (Owens et al, 1958; Miller et al, 1962; Caguin and Carter, 1963; Evans and Wellington, 1964; Hill et al, 1969; Danielson et al, 1970). Cerebral emboli of fibrin found by Hill et al (1969) may be a result of the platelet and leucocyte aggregation that occurs

in transfusion blood (Swank and Porter, 1963).

Pre- and post-operative sources of emboli include calcified valves (Gilman, 1965) and left atrial thrombi that form during recurrent episodes of fibrillation (Allen, 1963).

Experimental work

Behavioural experiments have been performed before and after extracorporeal circulation in rats (Popovic et al, 1967). Animals were conditioned to self-stimulate a pleasure response through an electrode implanted in the hypothalamus. The rate of self-stimulation was not significantly different following whole-body cooling to 16°C with a pump-oxygenator. This is not surprising, since the hypothalamus is only rarely damaged by extracorporeal circulation.

Hypotension of the cerebral blood vessels is a common feature of extracorporeal circulation. Cerebral blood flow is normally regulated by arterial carbon dioxide tension over a wide range of cerebral perfusion pressures (Lassen, 1964; Harper, 1965; Zwetnow, 1968), but there is some disagreement about the maintenance of vasomotor reactions to changes in P_{CO_2} during by-pass procedures. According to Wollman et al (1966) normal vasomotor reactions are maintained in man, but in dogs Ankeney and Viles (1961) and Horecky et al (1966) found that the rate of cerebral blood flow became entirely dependent upon cerebral perfusion pressure. It is unlikely that brain damage in extracorporeal circulation is caused by reduced cerebral blood flow alone. Whole-body oxygen consumption and tissue

PO_2 remain normal at total blood flow rates of 60-100 ml/kg body weight/min. (Anderson and Senning, 1958) and redistribution of blood flow preserves normal brain tissue PO_2 at total blood flow rates as low as 40 ml/kg body weight/min. in dogs (Schwartz et al, 1959).

Pathological changes in dog brain have been correlated with the duration of circulatory arrest (Johnston et al, 1966). The earliest signs of irreversible neuron damage were detected after 30 minutes of circulatory arrest at 13-20°C in dogs by Schweickert and Sickinger, (1960). They found ischaemic cell change of the Purkinje cells and neurons in the cerebral cortex. Minor, reversible neuron swelling, chromatolysis and oedema were the only microscopical changes observed in dog brains after 30 minutes of circulatory arrest at 7-8°C by Steegman and Miller (1965), or after 45 minutes with the oesophageal temperature drifting upwards from 6-8°C by Miller et al, (1965). After longer periods of arrest, neuron swelling and necrosis were accompanied by astroglial proliferation and oedema in the cerebellum, caudate nucleus and scattered cortical regions. Connolly et al (1965) found no brain damage after periods of circulatory arrest up to 1 hour at oesophageal temperatures of 2.5-3.0°C, but longer periods caused chronic, though subtle, demyelinization, scattered neuron loss, and increase in glial numbers. Kuchibaa (1966) reported biochemical changes suggesting hypoxic damage after 90 minutes of

circulatory arrest with an oesophageal temperature of 10°C. No histological changes were described although his plates appear to show some ischaemic cell change.

The rate of cooling was thought to be important by Lesage et al (1960). Circulatory arrest of 1 hour at oesophageal temperatures below 10°C was tolerated by 16 of 20 dogs. The four deaths resulted from large temperature gradients between organs after rapid cooling, and/or anaerobic metabolism. Williams (1964) also found that rapid cooling is harmful and recommended hemodilution, a high PCO_2, and deep anaesthesia to avoid vasospasm and blood sludging during cooling. Zingg and Cantor (1960) pointed out that the oesophageal temperature is a poor indication of brain temperature, the brain being up to 6°C warmer than the oesophagus during cooling. Furthermore, the brain is not uniformly cooled. Mid-brain temperature may be as much as 7°C lower than that of the cerebral cortex. Temperature gradients were also implicated by Almond et al (1964) who found that ischaemic necrosis of dog cerebellum, hippocampus, cerebral cortex and caudate nucleus is more severe after rapid cooling (water-blood gradient 20°C) than after slow cooling (water-blood gradient 4-6°C).

By examining cerebral biopsies at various stages during cooling and rewarming, Černý et al (1966) were able to evaluate the pathological effects of cooling per se. They found varicose thickenings of nerve fibres during the period of deep hypothermia (13-15°C oesophageal

temperature), but not at the end of rewarming. Slight chromatolysis of neurons was seen at this stage.

Cerebral oedema could result from damage to the blood-brain barrier by extracorporeal circulation (Dobell et al, 1966), circulatory arrest (Miller et al, 1965), air embolism, or the use of excess quantities of antifoam solution (Hodges et al, 1958).

Focal lesions of the type usually associated with circulatory emboli have been detected in the brains of dogs following extracorporeal circulation with pump-oxygenators (Edmunds et al, 1963). Various experimenters have identified the emboli as silicone, fat, platelets or air.

Solutions containing silicone are used in oxygenators to prevent foaming of the blood. The possibility of gas bubble embolization is thereby reduced, but it is replaced by the equal hazard of silicone embolization (Smith, 1960; Lindberg et al, 1961).

Fat emboli are well known in pump-oxygenator techniques and may cause morbidity and death (Owens et al, 1958; 1959; Lee et al, 1961). In addition to the fat liberated from tissue incisions, fat globules may be formed in blood as a result of deconjugation of membrane lipoproteins during blood-gas interface denaturation. Progressive deterioration of the lipoprotein capsules of chylomicrons releases lipid nuclei which coalesce to form globules (Adams et al, 1959; Lee et al, 1961). Increased blood viscosity is a further consequence which, it has been suggested, may cause sludging and occlusion of

the microcirculation (Lee et al, 1961; Wright et al, 1962). The incidence of fat globulemia and fat emboli is less when a membrane-type oxygenator is employed, and can be greatly reduced with disc and bubble oxygenators by adding a non-ionic detergent to the blood prime (Adams et al, 1959; Wright et al, 1963; Danielson et al, 1970).

Platelet aggregates have been identified in the heart, kidneys and lungs of patients who died after cardiopulmonary by-pass (Jenevein and Weiss, 1964). The rate of formation of platelet and leucocyte aggregates in blood stored in heparin or acid-citrate-dextrose (ACD) has been measured by the screen filtration pressure method of Swank and his colleagues (Swank, 1961; Hirsch et al, 1964; Johnson et al, 1967). Aggregates begin to form during the first 24 hours after collection (Swank and Porter, 1963). The process is marked in a small proportion of blood packs 2 days after collection, and all blood packs contain numerous aggregates after 10 days. When stored blood is transfused into cats or dogs, inadequate capillary perfusion is indicated by EEG changes, but the EEG remains normal if the blood is continuously filtered through Pyrex glass wool (Hirsch et al, 1964). During extracorporeal circulation, screen filtration pressure is highest (worst) at the beginning of by-pass and declines as the by-pass proceeds (Swank and Porter, 1963; Ashmore et al, 1968). The reduction in circulating aggregates is thought to be due to mechanical disaggregation by the pump and the filtering function of capillary

beds (Swank et al, 1963).

Platelet and leucocyte aggregates can be removed by filtering the blood through Pyrex glass wool, or any of several synthetic fibres such as Orlon or Dacron (Swank, 1961; Hirsch et al, 1964), but the platelet count is thereby reduced so that bleeding may become a post-operative problem. A better remedy would be to prevent aggregates forming. Ashmore et al, (1968) claim that aggregation occurs when the prime blood is mixed with that of the recipient, but the evidence presented in Swank's papers shows clearly that aggregation occurs even without mixing. Adenosine diphosphate (ADP) is well known to cause platelets to aggregate and it seems likely that platelets aggregate in stored blood in response to the high concentrations of ADP produced by metabolically active red cells (Bennett, 1968). Dipyrimidine compounds have recently been introduced to inhibit the uptake of adenosine by platelets, but they have not been acceptable for extracorporeal circulation because of their profound hypotensive effects (Didisheim et al, 1970).

Air may be admitted into the blood stream during intra-cardiac surgery, especially when the left ventricle is opened, but this can be avoided by careful surgery (Jaén et al, 1970), or by use of the aortic catheter described by Tsuji et al (1970). Gas bubbles formed in the circulating blood by pump-oxygenators present a greater problem. Bubble oxygenators are the worst culprits, while disc and

membrane oxygenators are less offensive in this respect (Jordan et al, 1958; Spencer et al, 1965; Selman et al, 1967; Aronstam et al, 1968; Patterson and Kessler, 1969). The rate of formation of gas bubbles is increased by agitating the venous reservoir, and by raising the flow rate of gases into the oxygenator (Patterson and Kessler, 1969). Raising the blood temperature is also liable to create gas bubbles. When the temperature of blood is raised, its PO_2 rises (Donald and Fellows, 1959). Macroscopic bubbles can be seen in circulating blood when a temperature differential of 17°C (20-37°C) is equilibrated in 6 minutes, though no bubbles emerge when the blood temperature is raised rapidly from 23°C to 37°C or when it is raised more slowly.

Microbubbles have been detected in circulating blood by the use of an ultrasound generator and transducer (Austen and Howry, 1965; Maroon et al, 1969; Patterson and Kessler, 1969). The number of echoes received by the transducer from microbubbles and particulate matter in the blood was only slightly reduced by incorporating a standard blood filter in the circuit, and because the velocity of ascent of bubbles is proportional to their diameters, microbubbles do not settle out in the debubbling chamber. Microbubbles may be formed even when an oxygenator is not employed (Willman et al, 1958; Bass and Longmore, 1969). A pressure drop occurs on the venous side of a pump roller as it compresses the cuff, and this causes gas to be

released from plasmatic solution. Liberated oxygen may be combined by haemoglobin but nitrogen forms microbubbles which are a hazard to capillary perfusion.

There are no reports of plastic embolizations as a result of extracorporeal circulation, though the possibility is suggested by the work of Rogers and Dunn (1969) who leached 454 mg. of plastic from polyvinyl chloride (PVC) tubing during a 48-hour test period.

Pulsatile and non-pulsatile blood flows

Pulsatile pumps have been constructed for physiological experiments (Dodrill et al, 1952; Wesolowski, 1954), isolated organ perfusion (Lindbergh et al, 1946; Hoff and Hayes, 1969), clinical left heart support (Goldman et al, 1966; Hinglais et al, 1967), experimental replacement of the heart (McCabe, 1959), and total by-pass during open-heart surgery (Dennis, 1956; Vaynrib et al, 1957; Nakayama et al, 1963).

Several reviewers attribute a significant physiological role to the pulse with very little direct experimental evidence (de Langen, 1958; 1959; Wilkens et al, 1962; Stead, 1965; Baust et al, 1968), but there is a strong case in favour of pulsatile blood flow that can be stated in teleological, theoretical, clinical and experimental terms.

The teleological evidence has been presented by Wilkens et al, (1962). The transition from aquatic to terrestrial environments

has been a critical episode in vertebrate evolution. Due to the loss of both water buoyancy and the protection provided by water against temperature fluctuations, metabolic demands are higher for animals living on land. This requirement has been satisfied in two stages. In the first stage developed by amphibians, lymph circulation was improved by the action of lymphatic hearts. Amphibians have cold blood and a low-pressure blood circulatory system. The second stage was the development of a high-pressure, warm-blood circulation in mammals and birds. At the same time the 3-chambered heart of amphibians and inefficient partial septa of reptiles have been replaced by 4-chambered hearts and lymphatic hearts were abandoned (Fig. B1). The increase in pulse pressure accompanying these changes is pronounced. For their schematic representation of cardiac evolution Wilkens et al (1962) unfortunately selected very specialised representatives. However, there may be some significance in the systolic pressure difference of 300% between frog and pigeon and a 400% higher pulse-pressure in the pigeon. By these devices, the tissues are supplied with nutrients and oxygen and the rate of movement of interstitial fluids is increased.

Further compartmentalisation has resulted in a second circulatory system containing lymph. Its functions are to return fluids to the blood and to transport large molecules such as plasma proteins and

lipoproteins which do not readily pass through capillary walls. If lymph flow is not adequate, these large molecules accumulate in the interstitial spaces and exert oncotic pressures that result in oedema. The monumental and diligent researches of McMaster and Parsons (1938) and Parsons and McMaster (1938) on the spread of dyes in isolated, perfused rabbit ears established that arterial pulsations are important for promoting the movement of interstitial fluids, and for the formation and flow of lymph.

Their conclusion is open to the criticism that results obtained from an isolated rabbit ear bear little application to total body circulation. Thoracic duct lymph flow is increased by 230-2900% during both partial and total by-pass (Baue et al, 1965), and Anabtawi et al (1966) found that thoracic duct lymph flow increases by 4 to 6-times initial flow rates after 30 to 40 minutes of extra-corporeal circulation, regardless of the type of blood flow. Although this has been used as evidence that non-pulsatile blood flow increases the rate of movement of interstitial fluids, the magnitude of the changes in thoracic duct lymph flow suggests a sizeable redistribution of body fluids from the blood to the tissues. Such an increase might result from blood stagnation in venous capillaries, so that increased thoracic duct lymph flow may be a consequence of inadequate tissue perfusion.

In the brain, the lymphatic system is represented by the

cerebral ventricles containing cerebospinal fluid (CSF). The arterial origin of CSF pressure pulsations is well documented (O'Connell, 1943; Bering, 1955; de Langen, 1959; Greenfield and Tindall, 1965; Dunbar et al, 1966; Guthrie et al, 1966; Laitinen, 1968), though a venous pulsatile component may be dominant under certain abnormal physiological conditions and sometimes under normal conditions (Hamit et al, 1965; Adolph et al, 1967; Dardenne et al, 1969).

The site of formation of CSF is generally believed to be in the cells of the choroid plexi, particularly those of the lateral ventricles (Miller and Woolam, 1962). The evidence for this belief is as follows:-

(a) Blocking the interventricular foramen of Monro increases CSF pressure within the lateral ventricles unless a major part of the choroid plexus is extirpated.

(b) Obstruction of the flow of CSF from the ventricles to the subarachnoid space raises intraventricular CSF pressure.

(c) When the lateral ventricles are exposed during neurosurgical operations, droplets of fluid are seen to form on the plexi.

Absorption of CSF is via the arachnoid villi into the large cranial venous sinuses and spinal extra-dural veins. By performing unilateral ventricular plexectomy and blocking the foramen of Monro with kaolin sponge, Bering (1955) was able to conclude that CSF

pressure pulsations originate in the arteries of the choroid plexi. He suggests that each arterial pulse acts as an unvalved pump ejecting CSF from the subarachnoid space through the arachnoid villi and into the venous system. Clinical support for this theory is provided by Dott and Gillingham (1958) who noted that cystic pouches filled with CSF in cases of hydrocephalus were associated with subarachnoid arteries. In one case, they observed one of these pouches being filled in a pulsatile manner, each pulse corresponding with cardiac systole. The importance of the choroidal arteries in the generation of CSF pulsations has been denied by Dunbar et al, (1966) and Guthrie et al (1966) who found the large arteries supplying the spinal cord to be the major influence, and by Laitinen (1968) who concluded that CSF pressure fluctuations are probably due to pulsations of the large arteries passing through the basal cisternae. It is important to resolve this problem of the origin of CSF pressure pulsations, because it is an important factor in deciding whether CSF is formed as an ultrafiltrate or as a secretion. But, even if arterial pulsations are unimportant in the formation of CSF, they may be responsible for its movement. This might explain the decline in the P_{O_2} of CSF during non-pulsatile perfusions presented in the discussion of the paper by Javid et al (1969).

If arterial pulsations are important in promoting the formation and flow of interstitial fluids, lymph and CSF, a theoretical case for pulsatile perfusion can be established. A reduced rate of flow

of interstitial fluids during non-pulsatile perfusion will soon result in oxygen deprivation of the tissues. The failure of the lymphatics to remove excess tissue fluids accumulating in the interstitial spaces will raise the interstitial fluid pressure and cause further reductions in the rate of transudation of plasmatic materials across the capillary walls. But, the interstitial fluid pressure will continue to increase and may eventually cause the thin-walled capillaries to collapse. Thereafter, blood will flow only in arterio-venous shunts. The process will be arrested by organ death as a result of generalised ischaemia. A mechanism such as this is implicated by the several reports of diffuse ischaemic cell changes in the brains of open-heart surgery patients.

The theoretical case for pulsatile blood flow is strengthened by the mathematical exposition of Shepard et al (1966), which predicts higher mean flow rates for pulsatile flows compared with non-pulsatile flows at equal mean blood pressures. The reason for this is that under a pulsatile regime, some of the lateral pressure energy component of total energy is used to expand the lumen of blood vessels. Due to the elastic recoil of vessel walls, this energy component is utilised to propel blood distally after peak systolic pressure has been reached.

Total energy is described by the term "energy equivalent pressure". For non-pulsatile flows, energy equivalent pressure equals mean blood pressure plus kinetic energy,

$$EEP = MP + KE,$$

but for pulsatile flows,

$$EEP \not> MP + KE.$$

Assuming negligible net gravitational energy, the difference between EEP and MP + KE for pulsatile blood flows is the capacitance of the vessel walls. Capacitance energy may be more important in the circulatory dynamics of the lung than in other organs since 20-30% of the energy supplied to the pulmonary circulation during right ventricular systole is stored by expansion of the pulmonary artery wall (Fishman, 1968).

This explanation partly accounts for the apparent increases in peripheral vascular resistance reported to occur during non-pulsatile perfusions (Nonoyama, 1960; Takeda, 1960; Nakayama, 1963; Burns et al, 1965; Mandelbaum and Burns, 1965; Mandelbaum et al, 1965; Jacobs et al, 1969), because capacitance energy is not included in the calculation of peripheral resistance according to the formula:-

$$\frac{\text{Arterial pressure (mm Hg) - Venous pressure (mm Hg)}}{\text{Blood flow (L/min)}}$$

However, it cannot be a complete explanation, because peripheral vascular resistance increases progressively with the duration of non-pulsatile by-pass.

Comparisons of changes in peripheral vascular resistance are complicated by the reflexogenic responses of carotid baroreceptors which respond to both static and pulsatile components of total energy (Ead et al, 1952; Filistovich et al, 1966; Giron et al, 1966; Koushampour and McGee, 1969; Soroff et al, 1969), and by the release

of vasoconstrictive humoural agents from the kidneys during non-pulsatile perfusions (Koehlstadt and Page, 1940; Many et al, 1968a; 1968b; 1969).

Pulsatile blood flow confers the advantage of agitating the diffusion shells of metabolic substances in the tissues and improving gas exchange. Metabolic studies have shown that the rate of tissue respiration is higher during pulsatile than during non-pulsatile perfusions (Nonoyama, 1960; Ida, 1962; Shepard and Kirklin, 1969; Trinkle et al, 1969). Capillary circulation is not uniform with non-pulsatile inputs because blood tends to flow in preferential arterio-venous channels (Ogata et al, 1960; Takeda, 1960).

Non-pulsatile blood flows are associated with a depression of kidney function not found during pulsatile perfusions (Hooker, 1910; Gesell, 1913; Judson and Rausch, 1957; Many et al, 1967; 1968a; 1968b; Jacobs et al, 1969; Paquet, 1969). Moderate to severe hydropic degeneration of kidney tubules is found only after non-pulsatile perfusions (Dalton et al, 1965a; 1965b), and unilateral renal artery depulsation results in increases in juxtaglomerular cell granulation and in tissue renin content that correlate with decreases in sodium and water excretion (Many et al, 1969).

In contradiction, Selkurt (1951) found no changes in plasma flow or filtration rate in kidneys perfused with a wide range of pulse pressure amplitudes. Depulsation of dog renal arteries produced no significant changes in the excretion rates of water and electrolytes,

and the clearances of inulin andparaminohippurate were unaffected (Goodyer and Glenn, 1951). The increase in peripheral vascular resistance alleged to be associated with non-pulsatile perfusion was not produced during perfusions of the kidneys by Ritter (1952) or Paquet (1965), though the latter author observed reduced tissue metabolism. Randall and Stacy (1954) detected only non-significant differences in blood flows in the hind legs of dogs perfused with pulsatile and non-pulsatile blood flows at equal mean blood pressures. Turbulence and vorticity are more apparent in pulsatile flows than in steady flows in vitro (Attinger, 1964).

Probably the most influential barrier to the adoption of pulsatile flows for extracorporeal circulation has been the researches of Wesolowski et al (1953). They found that non-pulsatile right-heart by-pass in dogs was compatible with normal respiratory functions and no permanent alterations in circulatory dynamics. This was confirmed by Clarke et al (1968) and by Wemple et al (1969). In the same series of experiments, Wesolowski produced preliminary evidence that pulsatile perfusion caused less systemic hypotension than non-pulsatile flows, but the point was subsequently negated by them finding no significant differences in hemodynamics, kidney function, vascular tone, recovery rate, blood indices and organ histology between pulsatile and non-pulsatile perfusions (Wesolowski et al, 1955). The criticism made against these results is that the use of high flow rates by Wesolowski

probably obviated the differences (Ogata et al, 1960).

Therefore, the evidence in favour of pulsatile perfusion is strong. What confusion remains may be largely due to the failure of some authors to define their concept of 'pulsatile blood flow'. Few reports provide example blood pressure and flow traces, and it is clear that some of the so-called "pulsatile" pumps deliver sinusoidal pressure and flow wave-forms which are totally different in form and effect from those generated by the heart. This criticism applies particularly to air-activated diaphragm and ventricle pumps such as those described by Dodrill et al (1952), Wesolowski (1954), Anabtawi et al (1966), Lindbergh et al (1966) and Wemple et al (1969). This may be an important reason for the failure of Anabtawi, Wesolowski and their associates to find significant differences between the effects of pulsatile and non-pulsatile perfusions.

When extracorporeal pumps have not been used, it has been necessary to pass the circulating blood through one or more depulsating chambers to simulate non-pulsatile perfusion. The introduction of arterial cannulae for this purpose can seriously prolong the systolic rise time on the arterial pulse contour distal to the damping chamber (Ead et al, 1952).

Conclusion

It is firmly established by neurological and pathological investigations that brain damage is a common sequel of open-heart

surgery. Autopsies of patients who died after open-heart operations provide convincing evidence of the patho-physiological effects of extracorporeal circulation. This has been verified by experiments on dogs, in which both ischaemic/hypoxic and embolic lesions have been produced.

The factors thought to be responsible for causing brain damage during extracorporeal circulation are hypotension, prolonged circulatory arrest, wide temperature gradients during cooling, cooling per se. and circulatory emboli of silicone, fat, blood cell aggregates and air.

The role of arterial pulsations in circulatory dynamics has been a controversial issue for many years, but the volume of direct experimental evidence is still small and mostly restricted to studies of kidney function. In this field, convincing evidence of the importance of the pulse in maintaining normal renal hemodynamics and kidney filtration characteristics has been accumulated. Some of this has been disputed, but there is no report in the literature that claims greater advantages for non-pulsatile blood flows in the kidneys or elsewhere.

In addition to the teleological belief that 'nature knows best', there are now theoretical reasons for expecting pulsatile blood flows to be beneficial. The aggregation of teleological, theoretical, clinical and experimental arguments in favour of pulsatile perfusion provides sufficient grounds for conviction. However, none of the

experimental reports on the effectiveness of pulsatile blood flows so far available has been concerned with brain pathology, despite the susceptibility of the brain to ischaemic/hypoxic damage. A survey of the literature makes it apparent that one reason for this is the difficulty of fixing the dog brain without causing artefacts that resemble early ischaemic cell change. Because of this, it has been necessary to allow animals to survive for several days before sacrifice, so that pathological changes become well developed. Unfortunately, it may also allow time for cell changes to be reversed. The transience of neurological sequelae in patients following open-heart surgery makes it important to detect these early changes.

The principal aim of these investigations has been to examine the effects of pulsatile and non-pulsatile blood flows upon nerve cells in the brains of dogs. As a logical development, the eradication of brain damage resulting from extracorporeal circulation has been adopted as a wider aim. Experiments designed to elucidate the second of these aims are currently in progress.

General and surgical procedures

Adult beagle and harrier dogs weighing 6.5-20.0 Kg were used for these experiments. General anaesthesia was induced by intravenous injection of 0.25-0.5g sodium thiopentone (Intraval). Following endotracheal intubation, the dogs were artificially ventilated by a Palmer Ideal respirator delivering 200-400 ml/stroke at 16 strokes/min., and light anaesthesia was maintained with a variable mixture of 0.5-2.0% halothane (Fluothane) in nitrous oxide and oxygen.

Clean surgical procedures were adopted for non-recovery experiments, and attempts to obtain recovery animals were performed under sterile conditions.

A modified Drew (1961) technique was used to establish an extracorporeal circulation, employing either occlusive DeBakey roller pumps for non-pulsatile blood flows (DeBakey, 1934), or a prototype pulsatile pump designed and constructed in the Stafford Estate Laboratories of General Electric Company Power Engineering Limited, Stafford, England. A technical description of this pump is enclosed as Appendix 1. The DeBakey pumps were the double roller type with rotor and stator surfaces both flat (Associated Electrical Industries Ltd.). The internal diameter of the silicone rubber cuffs was 6mm.

and the rotors were adjusted so that the cuffs were just occluded. The traverse of each roller was approximately 25 cm. and the stroke volume for a 180° turn of the rotor was approximately 10 ml.

The extracorporeal circuit is shown as a diagram in Figure C1. The circuit may be considered as an extension of the heart chambers with the atria continued into the reservoirs (RR and LR) and the function of the ventricles performed by the roller pumps (RP and LP). For pulsatile flows, the roller pumps were replaced by the Morton-Keele pump with Harvard remote heads. The circuit differs from that described by Drew (1961) in having the heat exchanger (HE) placed in the left atrial return instead of being in the delivery line from the left pump. This translocation was introduced to reduce damping of the pulse delivered by the pulsatile pump. The tubing was 6 mm. internal diameter PVC. The reservoirs were also made of PVC and each had a capacity of 300 ml. The heat exchanger consisted of 8 stainless steel tubes each 1.5 m. in length and with an internal diameter of 6 mm. mounted in a copper water jacket. Only 4 of these tubes were used and they were thoroughly cleaned and polished before sterilisation.

All of the cannulae were made of stainless steel. The atria were drained through 6mm. internal diameter fenestrated cannulae. The input cannula size was determined by the diameter of the artery. The internal diameters of the cannulae were 4.0 or 5.0 mm. for the pulmonary artery, 2.5, 3.0 or 3.5 mm. for the femoral artery, and 4.0, 5.0 or 6.0 mm. for the proximal aorta.

The extracorporeal circuit was primed with approximately 1300 ml. dog blood, 10 ml/Kg body weight 10% low molecular weight dextran in 0.9% sodium chloride (Rheomacrodex), plus 4 ml. of 10% Mannitol/Kg body weight, 4 ml. 20% sodium bicarbonate, 25 mg. heparin and 3 ml. 20% calcium chloride for each 500 ml. blood. One million units of penicillin were added for recovery experiments.

Via a median sternotomy, the pericardium was opened. Both atria were cannulated for venous drainage. The lungs were perfused by a cannula passing through the infundibulum of the right ventricle and pulmonary valve into the pulmonary artery. These cannulae were immobilised by nylon purse-string sutures. For non-pulsatile circulation, the systemic arteries were retrogradely perfused through the left femoral artery. The proximal aorta was cannulated for pulsatile flows because earlier experiments had shown that the pulse is severely damped when perfusing through narrow cannulae such as those necessary for femoral artery inputs. Aortic cannulation was achieved either directly or via the apex of the left ventricle, and the cannula was held in place by a nylon purse-string suture.

When all of the cannulae were in place, both right and left pumps were started at very low flow rates and the venous clamps removed. A standard pulse frequency of 100 pulses /min. was employed in pulsatile experiments. The pump flow rates were then gradually increased over a period of 5 minutes if possible until the estimated

flow rate of 75 ml./Kg. body weight/min. had been obtained. However, poor venous returns usually limited the maximum initial pump flow rate to 50-75 ml./Kg. body weight/min. When flows had been stabilised the ventricles of the heart were electrically fibrillated and maintained in a state of fibrillation by further D.C. shocks as required throughout the 1, 2 or 3 hours of perfusion. The ventricles were electrically defibrillated before weaning off the pumps.

The temperature of water in the heat exchanger was continuously regulated to maintain the oesophageal temperature between 29.0°C and 31.0°C. Cooling to this range of temperatures took 10-15 minutes. Rewarming was performed during the last 20 minutes of perfusion, and the oesophageal temperature was raised to 29.9 - 35.6°C at the end of this period. Pump blood flow rates were continuously adjusted to maintain steady levels of blood in the reservoirs and flow rates were recorded regularly at 10-minute intervals. Blood loss from surgical incisions was replaced by adding transfusion blood to the reservoirs. Arterial blood pressure, venous pressure, oesophageal temperature, electrocardiograph, and arterial blood carbon dioxide tension, pH, standard bicarbonate and base excess were monitored throughout the experiments. Details of the physiological recording techniques and results are presented in the section headed 'Physiological data'.

After defibrillation, non-recovery animals were perfuse-fixed by the technique described in Appendix 2. At this stage of recovery experiments, the surgical incisions were repaired with nylon sutures. A protamine titration was performed to determine the concentration of heparin remaining in the blood. The blood volume of the dog was estimated as 85 ml./Kg. body weight and 1 mg. protamine sulphate was administered via the venous drip to neutralise each 1 mg. heparin. Two chest drainage tubes were inserted and the sternum was closed with stainless-steel wire. Blood was given slowly by the intravenous drip. Ampicillin and Cloxacillin, 500 mg. of each, were given by intra-muscular injection and the animals were allowed to survive for 7 or 8 days if possible before perfusion-fixation.

Programme for histology experiments

Two series of experiments were performed for subsequent brain histology - non-recovery and recovery. Non-recovery animals were perfuse-fixed immediately after defibrillation following 1, 2 or 3 hours of extracorporeal circulation. Except for a 2-hour by-pass in experiment 86, the duration of extracorporeal circulation was 1 hour in all recovery experiments. This period was timed from the induction of ventricular fibrillation to the first attempted defibrillation. In some cases, the by-pass period was extended by up to 20 minutes

because of slow rates of rewarming or repeated defibrillation failures.

Four dogs served as controls. These were subjected to thoracotomy under general anaesthesia and immediately perfuse-fixed. A further control was obtained by maintaining dog 118 under general anaesthesia for 3 hours with the thoracic and pericardial cavities open before perfusion-fixation.

In Tables C1 and C2, the values for body weight were obtained immediately prior to surgery, and brain weight is the weight of the fixed brain including the rostral spinal cord sectioned between the second and third cervical segments, but excluding the anterior parts of the olfactory bulbs.

Experiment 154 is not included in these tables. A 10.9 Kg. beagle dog died approximately 12 hours after 1 hour of pulsatile flow extracorporeal circulation. The brain was removed 8 hours later and fixed by suspension in Cammermeyer's Susa for 1 week before processing.

Comments on experimental failures

Experimental failures are listed in Tables C3 and C4. The stage at which death occurred is stated under Stage of perfusion in Table C3 and under Stage of procedure in Table C4. The recovery period is timed from the end of perfusion. Of the 10 attempts to obtain 1 week recovery after pulsatile perfusion, only 2 reached a successful

conclusion. Of the 10 attempts to obtain 1 week recovery in the non-pulsatile recovery series, 3 dogs survived for 1 week, 3 experiments were terminated prematurely because of poor prognoses, and 4 dogs died during the early recovery period.

Fatal technical errors were committed in 4 experiments (numbers 89, 90, 138 and 150). The positioning of the aortic cannula for pulsatile perfusions is critical. Other experiments showed that the internal diameter of this cannula must be at least 4 mm. or the rise time on the arterial blood pressure curve will be extended to unacceptable values, i.e. longer than 20% of the pulse cycle. This requirement adds to the technical difficulty of cannulating narrow aortae and introduces the possibility of reduced coronary blood flow due to obstruction of the proximal aorta by the cannula. In experiment 150, this resulted in acute myocardial ischaemia and defibrillation failure at the end of the perfusion.

Conversely, if, when the aortic cannula is inserted via the apex of the left ventricle, it is not pushed through the aortic valve, it appears that back flow of blood through the incompetent mitral valve exerts back pressures upon the pulmonary veins and capillaries. Withdrawal of the aortic cannula into the left ventricle resulted in distension of the heart, severe pulmonary oedema and bronchial haemorrhage at the end of perfusion in experiment 155.

Distension of the heart can also occur by leak-back of blood

into the right ventricle from the pulmonary artery cannula. Right ventricular distension was associated with aspirated blood-stained bronchial fluid in one unsuccessful pulsatile recovery experiment (number 157), but it also occurred in two unsuccessful non-pulsatile recovery experiments with no subsequent lung damage (numbers 143 and 158). Blood-stained bronchial fluid was aspirated during perfusion in another eight experiments and in one experiment during the recovery period (Tables C5 and C6). Table C6 shows that only 1 animal survived for one week following the aspiration of blood-stained bronchial fluid.

The cause of this lung damage is not clear but some of the factors involved may be recognised. Measurements of blood pressure in the pulmonary artery during pulsatile perfusion were made during experiment 141 (Table C10). Compared with pressures produced in the pulmonary artery by the right ventricle, those generated by the pump had relatively more rapid rise times on the arterial blood pressure curves and the mean arterial blood pressure was higher. Since the pulsatile pump appears to eject blood too rapidly into the pulmonary artery, attempts have recently been made to lengthen the ejection phase of the right pump. However, lung damage also occurred during non-pulsatile perfusions. This may be explained by the increase in capillary permeability that is known to occur in the lungs during extracorporeal circulation (Kuwana, 1962), but another important factor is the age of the pump priming blood. In all experiments up to number 143, blood collected 24-48 hours previously was used to

prime the extracorporeal circuit. It was suspected that platelet aggregates forming during storage of the blood after collection might become pulmonary vascular emboli with intra-alveolar bleeding as a consequence (Swank and Edwards, 1968). A series of failures was arrested when blood collected on the morning of experiment number 144 was used to prime the circuit. This is an important modification of technique necessitated by repeated experimental failures but probably affecting the extent of cerebral embolization.

Nevertheless experimental failures have continued and there have been two further cases of blood-stained bronchial fluid formation (experiments 155 and 157) since the modification of technique was introduced. It is easy enough to find pathological signs at the post-mortem examination. The experimental procedure is both complex and hazardous even when the perfusion is uneventful, and some organic damage and biochemical disturbance is inevitable. But the pattern of morbid regression is fairly consistent. Animals either do not recover consciousness, suffer from prolonged post-operative bleeding into the thorax, fail to defibrillate, or make several hours of good progress and then experience a sudden cardiac arrest (Table C7).

Prolongation of the anaesthetic state and defibrillation failure suggest inadequate cerebral and myocardial perfusion during by-pass. Twelve animals failed to recover consciousness within the first 2 hours after by-pass. Only 2 of these were in the pulsatile group. All of

the 4 animals that recovered consciousness within the first 2 post-operative hours had received pulsatile perfusions. The heart would not defibrillate at the end of one pulsatile perfusion (experiment 150 referred to on page 36).

Cerebral and myocardial perfusion will be considered in a later section, but it is worth noting at this stage that Kubota (1968) measured reduced rates of cerebral blood flow in human patients undergoing extracorporeal circulation at 25-30°C. Focal subendocardial haemorrhages have been reported after extracorporeal circulation in dogs by Lev et al (1965) and Super et al (1967). The former authors examined biopsy specimens obtained before and during by-pass by electron-microscopy. They found an enlarged sub-sarcolemmal space containing occasional rounded mitochondria immediately after placement of the cannulae and becoming more diffuse after normothermic extracorporeal circulation with a pump-oxygenator.

Sudden cardiac arrests terminated 2 of the pulsatile recovery experiments (numbers 132 and 154) and 3 non-pulsatile recovery experiments (numbers 97, 142 and 158). Moderate bradycardia and tachycardia were frequent post-perfusion features. Neither of these abnormalities could be considered to be a preliminary warning of impending cardiac arrest. Table C8 shows heart frequencies immediately before cannulation and during chest closure for recovery experiments. Post-perfusion heart frequencies were lower than before perfusion in 4 of the 5 survivors. Of the 10 animals that died after perfusion,

5 had lower post-perfusion heart frequencies. Animals with high pre-perfusion heart frequencies tended to have lower heart frequencies after perfusion, and conversely, those with low pre-perfusion heart frequencies had higher heart frequencies in the early post-perfusion period. Pre-perfusion heart frequencies were between 100 and 246 beats/min. ($\bar{x}$ = 154.0, d = 39.251) and after perfusion the range was 60-210 beats/min. ($\bar{x}$ = 141.6, d = 40.854). These differences are not significant ($p > 0.1$).

Like defibrillation failure, sudden cardiac arrests may also result from inadequate myocardial perfusion during by-pass. The delayed response could be due to the latency in development of acidaemia that can follow systemic shock (Fowle, 1968), or to the continued release of myocardial enzymes such as hydroxybutyrate dehydrogenase that occurs for several days after extracorporeal circulation (Welbourn et al, 1966). Traumatic, haemorrhagic or transfusion shock is indicated by widespread haemorrhages in the viscera and splanchnic pallor with venous congestion ("homologous blood syndrome" of Litwak 1963). In this respect it may be significant that in comparing the abdominal aorta blood pressure changes during successful and unsuccessful recovery experiments, the blood pressures of the animals that died were lower at all stages from the start of recording to the end of the perfusions. This is further considered in the section on physiological data (Fig. C5).

Inadequate coronary perfusion during extracorporeal circulation can result in late cardiac arrhythmias and arrests. It is to be expected that the movements of myocardial tissue fluids are reduced in the same way as other tissue fluids during non-pulsatile perfusions. However, even with pulsatile flows, it is possible that myocardial tissue fluid movement is reduced because of the absence of coordinated cardiac muscle contractions with the ventricles fibrillated. The reduced tissue fluid movements may produce temporary ischaemic changes in the myocardium that are the cause of inadequate cardiac output in the early post-operative period. The vicious circle of low cardiac output and acidosis could eventually result in cardiac arrest.

High coronary perfusion pressures during extracorporeal circulation can also lead to low cardiac output in the early post-operative period because of reduced compliance of the cardiac muscle (Brown et al, 1969).

If coronary perfusion characteristics are responsible for post-operative cardiac arrests, it would probably be beneficial to provide the heart with more assistance during the early post-operative period by prolonging the period of weaning off the pumps.

Persistent post-operative bleeding has occurred on eight occasions. Only 2 animals in this category survived for 1 week (numbers 98 and 105). The rate of fluid drainage from the chest drainage tubes varied from 2 or 3 drops/min. to 7 ml/min. in the first 2 hours after skin closure. The range of total fluid volumes drained

from the chest during the post-operative period was 100-800 ml. This volume was always replaced by intravenous transfusion of blood as accurately as possible to maintain a constant effective blood volume as indicated by the maintenance of a normal central venous pressure. The duration of bleeding was from 30 mins. to 10 hours. Blood loss did not always determine survival or death. Dog number 98 tolerated a total fluid loss of 720 ml. at the rate of 6ml/min. while dog number 143 died after a total fluid loss of 240 ml. at 1ml/min. However, postoperative bleeding appears to have been a major factor in the early deaths of dogs 132, 155 and 157 (pulsatile series), and of dogs 86, 97 and 142 (non-pulsatile series).

Post-operative bleeding is thought to be a consequence of lung damage. Gross haemorrhages and increased alveolar dead space follow haemorrhagic shock and transfusions of stored blood in dogs (Swank and Edwards, 1968). These are avoided by the administration of a serotonin antagonist. Extravasation of red cells following extra-corporeal circulation in both dogs and humans is claimed by Melrose et al (1965) to be a graft against host reaction, the transfused red cells being the graft and the pulmonary capillary endothelium being the host tissue. A non-blood prime would be a protection against this immune reaction.

Of course, lung damage may be a consequence of left ventricular failure and redistribution of the blood volume to the pulmonary circulation.

Discussion

A review of the literature for survival rates and causes of death during and after extracorporeal circulation in dogs leaves the reviewer with a feeling of scepticism. Technical accidents are reported only in articles introducing new techniques (Wesolowski et al, 1952; Drew et al, 1959) and the failure rates are generally low. In most cases, important experimental information is not provided.

Thus, Aronstam et al (1968) claimed that 13 of their 15 dogs survived after 30 mins. of normothermic pump-oxygenator perfusion, but the survival time is imprecisely stated as "for several hours after cardiopulmonary bypass". Evidence of cerebral air embolism was detected in all of their dogs after death or sacrifice.

When circulatory arrest is established at low body temperatures, the duration of arrest is an important determinant of survival rates. Lesage et al (1960) recovered 2 dogs after circulatory arrest for 30 mins. at 7°C or less, but when the period of occlusion was extended to 60 mins., 4 of 20 dogs died with brain damage. Drew et al (1959) were able to produce only 2 surviving dogs in their initial series of 12 dogs cooled to a pharyngeal temperature of 10°C. Ten of the 12 dogs were subjected to circulatory arrest for 30 mins. at 10°C but the authors did not make it clear whether the survivors came from this group or from the remaining 2 dogs, that were rewarmed as soon as the

pharyngeal temperature reached 10°C. In a further four experiments with whole-body cooling to 15°C and circulatory arrest of 20-45 mins., 2 dogs survived with no neurological damage. Causes of death were thought to be cerebral damage, haemorrhage and prolonged anaesthesia. Johnston et al (1966) perfused 5 dogs by a modified Drew (1961) technique for 3 hours at 10°C. Three dogs survived longer than 1 week. The survival rate was reduced to 2 out of 5 after 1 hour of circulatory arrest at 15°C, and to 0 out of 6 after 3 periods of 1 hour circulatory arrest at 10°C.

A pulsatile pump was used by Wesolowski et al (1952) to obtain total by-pass for 1-2 hours in 17 dogs. Twelve of these were short-term survivors, but 7 of the 12 died with late post-operative infections. Pulsatile left heart by-pass for 12 hours was performed on 10 dogs by Jacobs et al (1969). All 10 dogs survived for 1 hour after which they were sacrificed. No temperature controls were maintained and the heart was allowed to beat during perfusion.

Several authors have compared survival rates after pulsatile and non-pulsatile perfusions. In the series of experiments reported by Wesolowski et al (1955), 3 dogs survived for more than 1 week after 140-189 mins. of pulsatile total by-pass and 7 of the 10 dogs perfused with non-pulsatile flows for 76-204 mins. also survived for longer than 1 week. The causes of death were bleeding, cardiac arrest and post-operative infection. Comparisons with their results are complicated by

their use of a homologous lung as an oxygenator and by their failure to fibrillate the heart during perfusion.

Two-hour total by-passes were established in dogs by Dalton et al (1965a) using either roller pumps or the Army Artificial Heart Pump to produce pulsatile flows. Of the 10 dogs in each group, 7 of the pulsatile group survived for 4 days but none of the animals perfused with non-pulsatile flows survived. Autopsies revealed cardiac dilatation, pulmonary and hepatic congestion, swollen kidneys and pleural and peritoneal serosal haemorrhages. In a second series reported by the same authors (1965b), 8 of 10 animals survived in each group of 3-hour perfusions with pulsatile or non-pulsatile blood flows. The major difference between the 2 groups was the ischaemic (hydropic) tubular necrosis found in the kidneys after non-pulsatile perfusions.

Trinkle et al (1969) employed pulsatile and non-pulsatile pumps in combination with a disc oxygenator to produce 2-hour total by-passes in 10 dogs (5 in each group). All 10 dogs survived, but they did not state whether the heart was fibrillated and the survival time was limited to 60 mins.

The duration of survival was not reported by Shepard and Kirklin (1969), nor did they state whether the heart was fibrillated or not. Total by-pass of 4 hours duration with a pulsatile pump produced 13 "long-term" survivors after 13 non-pulsatile perfusions, but only 4 of

the 10 dogs in the pulsatile group survived.

The discrepancies in reporting details of perfusion techniques and survival times make precise comparisons difficult, but it is clear that most investigators have produced higher survival rates than ours. Our results can be made comparable by shortening the accepted survival time from 1 week to 1 hour as Trinkle et al (1969) have done (Table C9). The survival rates are thereby increased from 20% to 60% for pulsatile experiments and from 30% to 90% for non-pulsatile experiments. This is obviously not a legitimate device but it facilitates comparisons.

Long-term survival rates produced by other research teams are higher than ours and it may be that this is due to their use of hemodilution primes and pump-oxygenators, thereby reducing lung damage and minimising surgical trauma. Of those who employed quadruple-cannulation techniques, the results of Wesolowski et al (1952) and Johnston et al (1966) are better, and those of Drew et al (1959) are worse than ours. Measures that are now being taken to improve the rate of survival include the pre-operative administration of antibiotics, hemodilution pump prime, and prolongation of the rewarming period with the heart beating. Preliminary results look hopeful.

PHYSIOLOGICAL DATA

Introduction

In the following section, physiological records obtained before, during and after perfusions are presented, analysed and related. The author of this thesis claims only to have collected the information together and to have assisted with the recording. Other members of the cardiology team, whose participation is acknowledged in the appropriate place, shared the technical duties of making the measurements and are responsible for their accuracy.

The analysis of physiological data has two objectives. The more important of these is to relate the physiological changes associated with pulsatile and non-pulsatile flows to the histological changes to be described in a subsequent section. Physiological changes occurring during perfusions and differences in physiological responses to pulsatile and non-pulsatile flows are assessed and statistically evaluated to determine their significance in the development of histological changes. Coincidental factors that may not be related to the flow systems are statistically assessed so that they can be eliminated as possible causative factors, or included as complications. The low arterial blood pressure that occurred before pulsatile perfusions may be cited as an example of a complicating factor.

The subordinate objective is to compare physiological changes occurring during perfusions with the results obtained by other investigators using similar flow systems but different pumps. This enables the results to be related to the flow systems rather than to the pumps.

Materials and Methods

Except for blood biochemical estimations, measurements were recorded approximately 10 mins. before, and every 10 mins. after the induction of ventricular fibrillation.

Arterial blood pressure (AP). Recordings of mean abdominal aorta blood pressure and pulse pressure contours were obtained from a number 5, 6, 7 or 8 French side-eye catheter passed into the lower abdominal aorta via the right femoral artery. The catheter was connected by stiff-walled nylon tubing to an Elema-Schönander 0-300mm Hg pressure transducer. Continuous monitoring was provided by an Airmec display oscilloscope and permanent records were made by an Elema-Schönander Mingograph 81 pen recorder. Pulmonary artery blood pressure was similarly recorded during experiment 141.

Blood flow. - The DeBakey pumps used for non-pulsatile perfusions were calibrated by the frequency of roller revolutions. For pulsatile flow recordings, a Medelec electromagnetic blood flow recorder was used with the in-line flow probe placed in the venous return from the left atrium to the left reservoir. The calibrations of both types of flow recorder were checked by pumping saline into a graduated measuring cylinder before use. A small error in comparisons was introduced by blood pooling in the animals but this was probably much less than the intrinsic inaccuracy of the instruments which is up to 12%.

Venous pressure (VP). - A slow saline drip was introduced into the

right cephalic vein by a 17-guage intravenous needle. The drip tubing was equipped with a 3-way tap so that a saline manometer could be added to record venous pressure.

Oesophageal temperature ($T^{o}C$) - A thermocouple passed into the oesophagus was connected to a multichannel temperature recorder for continuous monitoring of oesophageal temperature.

Arterial carbon dioxide tension ($P_{a_{CO_2}}$). - Blood samples were obtained from the femoral artery before commencing perfusion and from a 1-way tap in the circuit between the left atrium and the left reservoir at 20-minute intervals during perfusion. Micro-Astrup equipment (Radiometer Ltd.) was used to measure $P_{a_{CO_2}}$, base excess and standard bicarbonate (Astrup et al, 1960; Siggaard-Andersen and Engel, 1960; Siggaard-Andersen et al, 1960). The values obtained were used as guides to the respiratory and metabolic states of the animals. Oxygen, carbon dioxide and sodium bicarbonate were administered accordingly and tidal volume was adjusted in an attempt to maintain a $P_{a_{CO_2}}$ of 40mm Hg.

Cerebrospinal fluid pressure (CSFP) - Attempts to record CSFP by catheterising the cisterna magna were jeopardised by artefactual results caused by the mechanical damage of small blood vessels in the leptomeninges. Such attempts were made in experiments 93, 94, 111, 112, 120 and 122. Catheterization was performed by puncturing the dura mater over the cisterna magna with a number 16 Tuohy needle,

and threading an epidural catheter through the Tuohy needle into the cisterna magna. The other end of the catheter was connected to a saline-filled extension tube of an Elema-Schonander 0-30mm Hg pressure transducer. Continuous monitoring and permanent records were obtained in the same way as for blood pressure.

Statistical analyses were performed according to the methods described by Bailey (1959) using an Industria Macchine Elettroniche IME86S digital computer. The values computed were arithmetic mean averages ($\bar{x}$, $\bar{y}$), standard deviation (d), correlation coefficient (r_{xy}), coefficient of regression (b_{yx}), points of intercept (a_{xy}, a_{yx}), student's coefficient of significance (t), probability (p) and linear regression ($y = a + bx$). Regression lines were constructed from the values $\bar{x}$, $\bar{y}$, b_{yx} and a_{yx}.

Results and comments

Figures C2 to C19 are based upon mean average measurements made during each group of experiments. The abbreviations 'P', 'NP' and 'A' refer to pulsatile, non-pulsatile and anaesthesia experiments respectively, and the numbers preceding these symbols indicate the numbers of experiments from which the mean values were calculated. The additional symbols '..S' and '..US' refer to successful and unsuccessful recovery experiments. Statistical evaluations of arithmetic means, standard deviations, students t probabilities, and correlation and regression coefficients are listed in Tables C11

to C24. The relationships between AP and other physiological variables and between peripheral vascular resistance (PVR) and other physiological variables are illustrated by the regression lines for individual experiments in Figs. C20 to C45.

The arithmetic mean value for pre-perfusion AP is low because the thorax had been opened and cannulation was in progress before the AP catheter was inserted into the abdominal aorta. The delay was necessitated by the risk of platelet thrombus formation at the tip of the catheter if the catheter had been inserted before the blood had been rendered incoagulable. An anticoagulant (heparin) was administered only when all of the surgical incisions had been completed and haemostasis had been secured. No values are provided for zero time because manipulation of the heart, adjustment of pump flows and venous drainage, and the fibrillation procedure caused transient, artificial changes in arterial blood pressure.

Figure C2 shows typical abdominal aorta blood pressure traces provided by the heart and by the pumps. It is important to emphasise the differences in form of the pulse contours produced in the arteries by the two pumps. The pulsatile pump produces a pulse profile that closely resembles the normal pressure pulse generated in the abdominal aorta by contraction of the left ventricle. A rapid upstroke is thought to be critical. In these experiments, the upstroke time was within the range 0.08 - 0.15 s. at 100 pump strokes/min. The

diastolic phase of the pressure pulse is probably determined by the elastic properties of the walls of blood vessels, especially those of elastic arteries and arterioles. Pulse pressures were within the range 25 - 90mm Hg.

Roller pump action is characterised by a pressure plateau near the mean value and sudden low-amplitude pressure reductions as each roller leaves the arterial end of the cuff. The range of pulse pressures is normally 2-15mm Hg but occasionally reaches 30mm Hg with very small dogs. The non-pulsatile 'pulse' contour has a prolonged upstroke phase occupying about 80% of the total 'pressure-pulse' cycle. During the remaining 20% of the cycle, a sharp reduction in blood pressure is followed by a recovery of approximately equal amplitude. Sometimes the recovery curve overshoots the mean value to produce a sharp peak resembling a systolic peak, but it probably represents a secondary harmonic produced in the arteries, the pressure catheter, or the pressure transducer.

It is important to note that the tip of the pressure catheter was located in approximately the same position in the lower abdominal aorta for both pulsatile and non-pulsatile inputs. However, the non-pulsatile input cannula was placed in the left femoral artery, so that the pressure contours recorded are those close to the point of ejection. Since the aorta is wider proximal to the heart than in the abdominal region, the non-pulsatile 'pulse' is likely to be

severely damped at the level of the circle of Willis. This amendment does not apply to pulsatile inputs because the input cannula was placed in the proximal aorta.

Although we have not recorded flow profiles produced by the roller pumps, the ejection phase may be considered to be almost continuous. So that, although the pumps do not produce entirely pulseless pressure contours, the elastic properties of the blood vessel walls are not employed in energy storage and the flows may be described as non-pulsatile according to the concept of "energy equivalent pressure". Therefore, use of the terms 'pulsatile' and 'non-pulsatile' is justified by consideration of flow dynamics and provides an easy descriptive contrast for future references to the flow systems.

Table C10 lists abdominal aorta and pulmonary artery pulse pressure values before and during extracorporeal circulation with the pulsatile pump. Compared with peak-to-peak systolic-diastolic pressures before perfusion, the pulsatile pump produced lower systolic-diastolic pressures in both the abdominal aorta and pulmonary artery, but whereas the mean pressure was lower in the abdominal aorta, it was higher in the pulmonary artery. The proportion of the pressure pulse cycle occupied by the upstroke was reduced in both arteries because of a lower pulse frequency and shorter upstroke times compared with pre-perfusion values. The relatively more rapid upstroke in the abdominal aorta may be of little consequence, but

a more rapid input to the pulmonary artery may account for the higher mean pulmonary artery blood pressures recorded during perfusion because kinetic energy is relatively more important in the low pressure pulmonary circulation.

A comparison of mean average APs during pulsatile and non-pulsatile perfusions is illustrated in Figs. C3 and C4. During cannulation severe hypotension developed in dogs of both groups, but the pressure reductions were greater in the pulsatile group. A fall in blood pressure at this stage is due to a small amount of blood loss, to embarrassment of the heart by manipulation and to the obstruction of blood flows into and out of the heart by the cannulae.

Cannulation of the proximal aorta for pulsatile perfusions is much more disturbing to cardiac function than femoral artery cannulation and this accounts for the more precipitous reduction in mean AP in the pulsatile group. The degree of this initial hypotension correlates with survival (Fig. C5). Animals died during the early post-operative period whenever the pre-perfusion AP fell below 60mm Hg in the non-pulsatile group, or below 45mm Hg in the pulsatile group. The inference of this is that, at a certain critical level of hypotension, irreversible physiological and/or metabolic changes occur and that the threshold value of AP is lower preceding pulsatile perfusions than before non-pulsatile perfusions. The influence of the duration of hypotension before perfusion commenced cannot be assessed because it has not been measured.

In the group of animals subjected to thoracotomy and halothane anaesthesia for 3 hours, the gradual lowering of mean AP correlated with the duration of anaesthesia, with VP, and sometimes with $T^{o}C$ (Figs. C12, C20, C21 and C22; Tables C13 and C14). In the pulsatile group of experiments, the mean AP showed a tendency to fall throughout 3-hour perfusions (Fig. C21). This resembled the gradual hypotension produced by 3 hours of anaesthesia alone (cf. Figs. C20 and C24). In contrast, mean AP increased rapidly during non-pulsatile perfusions, reaching a peak after 60 mins. (Figs. C4 and C22). The mean AP changes in both experimental groups were not correlated with changes in VP, $T^{o}C$, Pa_{CO_2}, or in pump blood flow rates (Figs. C6 to C11 and C24 to C33; Tables C13 and C14). The correlations between AP and other physiological variables are considered to approach a normal bivariate distribution. If this is not so, the regression coefficients are more appropriate than the correlation coefficients and the levels of significance are lower (Tables C15 and C16).

Spontaneous defibrillation occurred repeatedly during some experiments, particularly when pulsatile perfusion was employed. Presumably this was due to the mechanical stimulation of the myocardium by pulsatile flows, and it may be considered to indicate satisfactory coronary perfusion. The heart was always refibrillated immediately, except when spontaneous defibrillation occurred during the last 5 mins. of perfusion. On these occasions, it was considered

to be expedient to permit the heart to beat rather than to refibrillate and have to electrically defibrillate within 2 or 3 mins. Because this situation arose in some pulsatile recovery experiments, it accounts for the rise in mean AP at the end of 1-hour perfusions (Fig. C3), which was not evident during the 3-hour non-recovery series (Fig. C4).

Fig. C13 appears to suggest that during the last 30 mins. of 1-hour pulsatile perfusions, blood flow rate from the pump declined while mean AP was rising. The significance of this is not clear and it was not verified during the 3-hour experiments (Figs. C10 and C14).

Venous pressure changes were unexciting, but there was a slight tendency for VP to rise within normal limits during 1-hour pulsatile perfusions (Fig. C6).

Peripheral vascular resistance was calculated using the formula:-

$$PVR = \frac{AP\ (mm\ Hg) - VP\ (mm\ Hg)}{Pump\ flow\ rate\ (ml/s)}$$

taken from Burton (1965). Changes in PVR are illustrated in Figs. C15, C16 and C17. The slight rise in PVR at the end of both pulsatile and non-pulsatile 1-hour perfusions was again due to occasional spontaneous defibrillation during the last 5 mins. of perfusion. In both the 1-hour and 3-hour experiments using non-pulsatile flows, PVR tended to rise during the first hour reaching a peak in 60-150 mins. during the 3-hour perfusions and declining slowly thereafter. The trend was similar in the 3-hour pulsatile perfusions except

that PVR declined during the first 40 mins. and declined more steeply after reaching its highest value at 90 mins. However, this conclusion is based upon only two experiments because of incomplete records for other 3-hour pulsatile perfusions. During the 1-hour pulsatile experiments, PVR was remarkably constant throughout the period of fibrillation.

Changes in PVR were correlated with AP changes (Tables C17 and C18). This is verified by regression analysis (Tables C19 and C20; Figs. C35 and C41). No correlations were established between PVR and VP, T°C or pump flow rates (Figs. C36, C37 and C39 pulsatile; Figs. C42, C43 and C45 non-pulsatile). The apparent negative correlation between PVR and $P_{a_{CO_2}}$ was not statistically significant (Fig. C38 pulsatile; Fig. C44 non-pulsatile. Tables C17 and C18).

As Figs. C13 and C14 show, the target flow rate of 75 ml/Kg body weight/min. was not achieved in these experiments. The mean values for pulsatile perfusions were 66.8 ml/Kg/min. (d = 5.46) in the 1-hour experiments and 56.4 ml/Kg/min. (d = 6.0) in the 3-hour experiments. For non-pulsatile perfusions, the mean values were 67.1 ml/Kg/min. (d = 1.76) and 64.4 ml/Kg/min. (d = 3.0) respectively. These differences were significant only for the 3-hour perfusions ($P < 0.02$). Blood loss was not meticulously replaced by transfusion but the rates of bleeding were insufficient to account for widely divergent and decreasing pump flow rates. These are dependent upon the flow of blood into the reservoirs from the atria.

Changes in CSFP are shown in Figs. C18 and C19. Accurate recordings of CSFP were obtained in experiments 94 and 112. In experiment 93, a drift in the zero calibration of the pressure transducer spoiled the results. Steady pressure values with no fluctuations corresponding to heart beat and respiratory movements were signs of occlusion of the catheter tip in experiment 122.

Both of the accurate records were obtained during 3-hour non-pulsatile experiments. Fig. C18 shows that in experiment 94, CSFP remained within normal limits for the first 2 hours, but then a sharp rise in CSFP to 60mm Hg followed a few minutes after an increase in AP. The CSFP declined after 5 mins. but remained higher than previously. No similar changes occurred during experiment 112 in which CSFP was maintained within the range 1-18mm Hg (Fig. C19).

When the brain was removed, much blood was evident beneath the arachnoid membrane in all 6 experiments in which attempts had been made to record CSFP. Blood probably accumulated in the subarachnoid space following damage to small blood vessels in the leptomeninges during catheterisation. The large rise in CSFP during experiment 94 may have been due to the rupture of a damaged artery in the sub-arachnoid space caused by the rise in arterial blood pressure a few minutes earlier.

Fluctuations in CSFP were synchronised with respiratory movements, heart rate and pump action. Pre-perfusion CSFP fluctuations corresponding

to heart action were 0.2 to 2.8mm Hg in amplitude in the non-pulsatile experiments, and 1-2mm Hg in the pulsatile experiment. During by-pass, the range of CSFP fluctuations was 0.1-0.6mm Hg with non-pulsatile flows, and remained between 1 and 2mm Hg with pulsatile flows. Complete records were not obtained during the pulsatile experiment.

Discussion

Definite conclusions can be drawn from the physiological data only with caution. The accuracy of the pulsatile flow recorder is questionable. Despite electronic integration, needle deflections with each pulse introduced a possible error in reading of up to 5% (up to 55 ml/min.). Furthermore, results obtained during *in vitro* experiments showed that an upward drift of as much as 12% could occur during 5 hours of continuous flow recording. A margin for error of up to 12% should be allowed for all flow measurements.

A second criticism is that VP measurements are not necessarily the same as central venous pressure measurements. Collapse of the right atrial wall around the atrial cannula can produce erroneous values for VP when recording from a peripheral vein.

Thirdly, complete results were not obtained for all experiments. The graphs have been calculated only from complete records.

Finally, the inherent fallacies of statistical evaluation must be recognised. These are:

1. Extrapolation from measured values to predicted values is not legitimate.

2. Statistical correlations do not establish cause and effect relationships.

3. Failure to establish a statistical correlation does not prove the absence of one, especially when the numbers of experiments are low or the variation is large.

Bearing these criticisms in mind, the following conclusions may be justified:-

1. The degree of hypotension incurred during the cannulation procedure may be critical in recovery experiments. Cannulation of the proximal aorta for pulsatile perfusions caused more severe hypotension than cannulation of the femoral artery for non-pulsatile perfusions, but it was better tolerated.

2. Abdominal aorta blood pressure tended to fall throughout 3-hour periods of general anaesthesia following thoracotomy. A similar tendency was observed during pulsatile perfusions, but with non-pulsatile flows AP increased during the first hour and remained high for the remaining 2 hours.

3. Peripheral vascular resistance was higher during non-pulsatile than during pulsatile perfusions. Changes in PVR during perfusions appeared to correlate with AP fluctuations.

4. Venous returns were higher during non-pulsatile than during pulsatile perfusions. This permitted higher pump blood flow rates towards the end of non-pulsatile perfusions in spite of elevated PVRs.

5. There was a tendency for P_{aCO_2} to rise during all perfusions and this was most marked in the 3-hour pulsatile group.

6. Arterial pulsations are transmitted to the CSF at the level of the cisterna magna. No general trend for CSFP to rise or fall was detected in 2 non-pulsatile experiments of 3 hours duration.

The severe hypotension that developed before most perfusions has only one comparison in the literature. After completing the cannulations for pump-oxygenator perfusions of dogs, Anderson and Senning (1958) found that AP had fallen from a mean value of 125mm Hg before thoracotomy to 90.5mm Hg after thoracotomy. It has not been the usual practice to fibrillate the heart during perfusions, so this may partly account for the maintenance of high APs by other groups of investigators. Peripheral artery cannulation also reduces the initial hypotension compared with cannulation of the proximal aorta. Ida (1962) avoided hypotension by cannulating the proximal aorta via one carotid artery, but he was not concerned to maintain cerebral blood flow. Reductions in AP from mean values of 113mm Hg to 94mm Hg (non-pulsatile) and of 114mm Hg to 77mm Hg (pulsatile) were measured during whole-body perfusions of calves by Shepard and Kirklin (1969) but the times of onset of these changes were not reported. They cannulated the proximal aorta via a woven Teflon graft to avoid pulse damping by narrow cannulae.

The hypotension that developed immediately prior to the commencement of extracorporeal circulation in our experiments, appears to be an inevitable consequence of cardiac manipulation and of placing cannulae in the heart. Other experimenters may not have observed the rapidly declining blood pressure because they failed to make frequent AP measurements at this stage, or they may have avoided hypotension by using different cannulation techniques. The profound effects of cannulating the left atrium are avoided by pump-oxygenator techniques.

It was noted that the hypotension that developed during cannulation for attempted recovery experiments was more severe in the animals that died than in those that recovered. The difference between successful and unsuccessful recovery experiments with respect to initial hypotension was significant at the 5% probability level in the pulsatile group though not in the non-pulsatile group (Table C24). The subsequent APs and PVRs during perfusions in these experiments were related to the severity of the initial hypotension. This indicates a persistent physiological consequence of initial hypotension but it would not be wise to hypothesise about the nature of this reaction, except to note that capillaries can collapse at low arterial pressures (Burton, 1954).

The gradual lowering of mean AP during 3 hours of anaesthesia alone was correlated with the duration of anaesthesia, VP and, in 2 of the 4 experiments, with T°C. Hypotension is a well-known effect

of halothane anaesthesia (Raventós, 1956). Since halothane was administered continuously in these experiments, hypotension probably resulted from the accumulation of halothane in the tissues. Decreasing body temperature during the anaesthetic period probably contributed to the hypotension because of a consequential reduction in cardiac output (Shumacker, 1960). The cause and effect relationship between AP and VP was not established.

Halothane accumulation might explain the hypotension that occurred during 3 hours of pulsatile extracorporeal circulation, but the relationship was less obvious in these experiments because halothane was administered only intermittently as required. The hypotensive effect of halothane anaesthesia was not apparent during the non-pulsatile experiments, in which AP increased during the first hour and remained high until the end of perfusion. Similar results were obtained during non-pulsatile perfusions by Nakayama et al (1963) and by Mandelbaum et al (1965). Changes in AP during our non-pulsatile experiments were not correlated with any of the other physiological variables measured, but they were correlated with changes in PVR. Therefore, increasing AP may be caused by increasing PVR but it would be fallacious to form a definite conclusion about this relationship because AP is included in the calculation of PVR.

Raised PVRs during whole-body perfusions of dogs have previously

been reported by Nonoyama (1960), Nakayama et al (1963), Burns et al (1965), Jacobs et al (1969), Shepard and Kirklin (1969) and Trinkle et al (1969). Wesolowski et al (1955) found no differences in PVR between dogs perfused with pulsatile and non-pulsatile blood flows, but their results have been criticised by Ogata et al (1960) because very high flow rates were employed (approximately 130 ml/Kg body weight/min. for dogs weighing 14 Kg).

It is generally assumed that raised PVRs during non-pulsatile perfusions are due to inadequate perfusion of the true capillaries as observed during microscopic examination of the omentum by Ogata et al (1960) and by Takeda (1960). The effects of pulsatile and non-pulsatile perfusions upon catecholamine levels were studied by Shepard and Kirklin (1969). They found no significant differences that might account for the higher PVRs of the non-pulsatile group. Burns et al (1965) and Mandelbaum and Burns (1965) calculated increases of 25% and 27% respectively for systemic and pulmonary PVRs during non-pulsatile perfusions compared with pulsatile perfusions. The increases were not prevented by sympathetic or parasympathetic blockade, nephrectomy or baroreceptor denervation. The claim that baroreceptors are not involved in PVR changes was disputed by Soroff et al (1969), who found that selective depulsation of the aortic arch and brachiocephalic trunk in dogs caused a 10.9% rise in PVR due to elevated systemic AP. Although Parsons and McMaster (1938)

and McMaster and Parsons (1938) detected reduced tissue fluid movements and lymph flows during non-pulsatile perfusions of rabbit ears, no attempts have been made to measure interstitial fluid pressures during whole-body perfusions. However, Takeda (1960) produced more oedema and higher capillary blood pressures during non-pulsatile perfusions compared with pulsatile perfusions.

Therefore, from the experimental evidence currently available, it would appear that differences in PVR during pulsatile and non-pulsatile perfusions may be due to the formation of preferential channels, to reduced tissue fluid movements, and possibly to baroreceptor reflex responses to non-pulsatile blood flows.

Alternatively, the differences in PVR can be explained in hemodynamic terms. Because PVR cannot be measured directly, it is derived as a function of arterial and venous blood pressures and blood flow rate. As explained by Shepard et al (1966), energy equivalent pressure is a more appropriate parameter than mean AP. Energy equivalent pressure is higher for pulsatile than for non-pulsatile flows at equal mean APs. Thus, the differences in PVR during pulsatile and non-pulsatile perfusions may be more mathematical than real, but changes in PVR developing during the course of perfusions cannot be due to mathematical fallacies. In the experiments reported here, PVR increased by 13.4% during 3 hours of non-pulsatile perfusion and decreased during pulsatile perfusions by 58.9%. These differences are highly significant (Table C21).

The poor venous returns obtained during pulsatile perfusions were in contrast to those obtained by Noncyama (1960), Ogata et al (1960), Nakayama et al (1963), Shepard and Kirklin (1969) and Trinkle et al (1969). They found venous returns to be more rapid during pulsatile than during non-pulsatile perfusions. Anderson et al (1967) have suggested that pulsatile pumps that suck blood from a reservoir into the pump head can redistribute blood from the systemic to the pulmonary bed. An imbalance between the left and right pumps can be maintained by expansion of the pulmonary blood volume at the expense of the systemic blood volume. The increase in pulmonary artery pressure, decrease in systemic AP and high incidence of lung damage in our experiments provide support for this thesis.

The more rapid rise in P_{aCO_2} during pulsatile perfusions provides additional support. Shepard and Kirklin (1969) noted a rise in P_{aCO_2} during non-pulsatile perfusions of calves but thought this was due to overventilation of the lungs before perfusion. The difference was less marked and not significant in the pulsatile group. However, their results are not readily comparable because they made only three measurements of P_{aCO_2} - before, during and after by-pass. The higher values of P_{aCO_2} in our pulsatile group could be due to less efficient respiratory gas exchange which may be due to the damage of lung capillaries by the rapid pump ejection or by high capillary blood pressures. Of course, changes in P_{aCO_2} can also be due to inadequate control of ventilation.

Measurements of CSFP were disappointing. The range of CSFPs during 2 non-pulsatile perfusions of 3 hours duration was 3-60mm Hg in experiment 94 and 1-18mm Hg in experiment 112. The highest values may have been due to subarachnoid haemorrhage. Bering (1955) measured cisternal CSFPs in 55 anaesthetised dogs. The mean value was 7.6mm Hg and the range was 3.5-11.2mm Hg. Similar results were obtained by Dunbar et al (1966) and Symbas et al (1967). Pulse pressures recorded during a pulsatile perfusion resembled those reported by Dunbar et al (1966) for normal dogs.

There have been no previous attempts to record CSFP during extracorporeal circulation. Such measurements could form an important part in the theory of pulsatile flow, but, due to technical problems, the experiments reported here were inconclusive.

Conclusions

Measurements of AP, VP, T°C, pump blood flow rate, blood indices and CSFP were made during pulsatile and non-pulsatile perfusions. The most significant difference between the two groups of animals was the lower AP-dependent PVR during pulsatile perfusions of up to 3 hours duration. Compared with non-pulsatile perfusions the hypotension that developed during cannulation was more severe, venous returns were lower and Pa_{CO_2} values were higher during pulsatile perfusions.

The relationship between physiological and histological changes will be examined in discussing the histology results.

HISTOLOGY EXPERIMENTS

Terminology

In this and subsequent sections, non-pyramidal nerve cells in the cerebral cortex are called 'cortical nerve cells'.

Materials and Methods

The perfusion-fixation technique and tissue processing schedule are described in Appendix 2. Paraffin wax sections were cut at thicknesses of 7-8μ for routine staining with Ehrlick's haematoxylin and eosin (H-E), Weigert's haematoxylin and Van Gieson (H-VG), and haemalum and periodic acid Schiff (H-PAS), and at 15μ for staining with luxol fast blue and cresyl fast violet (LFB-CFV) and cresyl fast violet alone (CFV). Some 7-8μ thick sections were stained with Mallory's phosphotungstic acid and haematoxylin (PTAH). Frozen sections 10-12μ thick were cut from the parietal region of the cerebral cortex and from the thalamus including the internal capsule. These sections were stained for fat with a saturated aqueous solution of Sudan III plus IV.

A note on the Nissl formations of normal nerve cells in the dog brain.

Nissl formations vary considerably between different nerve cell types and between nerve cells in different regions of the dog brain (Figs. C46, to C51). Peripheral clumps of Nissl substance are seen in the globus pallidus and supraoptic nucleus. Peripheral

rims of Nissl substance may also be found in some cortical nerve cells though not in pyramidal neurons. However, the Nissl substance is more usua-ly uniformly distributed except for a higher concentration in the axon hillock of nerve cells in the cerebral cortex. Dense clumps of Nissl substance occur in some cells of the thalamic nuclei, red nucleus and giant-celled reticular nucleus, and elongated clumps of Nissl substance form densely-staining bands in the nerve cells of the substantia nigra, dorsal vagal nucleus and inferior olivary nucleus. Less dense bands of Nissl substance occur in the nerve cells of the hippocampus and amygdaloid nuclei. The majority of neurons in the caudate nucleus, putamen, claustrum and thalamus are only slightly stained with CFV and appear to have very little Nissl substance.

These normal Nissl formations must not be confused with pathological cell changes such as central and peripheral chromatolysis.

The assessment of pathological changes in nerve cells following ischaemia and/or hypoxia.

Classical definitions of pathological cell changes are based upon histological examinations of human brains fixed by immersion in formaldehyde. 'Normal' nerve cells described and figured in this thesis differ considerably from the classical 'normal'. However, the classical categories listed by Greenfield and Meyer (1963) appear to be appropriate. Three types of nerve cell change have been

attributed to ischaemia and/or hypoxia:

Nissl's acute cell disease (Spielmeyer's acute cell swelling) (ACS). Acute cell swelling appears as an early nerve cell reaction. The nucleus, cell body and processes all swell. The nucleus appears to swell first and there is some loss of nuclear chromatin. Swelling of the perikaryon is accompanied by diffuse staining of the cytoplasm and usually the periphery of the cell appears to be empty. Rarefied neuropil in the vicinity of swollen nerve cells appears to be composed of distended astrocyte and cell processes. Acute cell swelling may be completely reversible or it may progress to ischaemic cell change or cell loss.

Ischaemic cell change (ICC). Shrinkage of the cell body is seen more frequently than swelling after ischaemic or hypoxic episodes. In nerve cells undergoing ICC, the cell body stains darkly and homogeneously with CFV and becomes eosinphilic. The cell processes are also shrunken and dark-staining and they become tortuous. The nucleus shrinks and stains darkly with CFV. In the pyramidal cells of the cerebral cortex, the nucleus may become triangular in shape but, due to the dark-staining cytoplasm, the nuclear membrane is frequently indistinct. Pericellular clear spaces may be the result of shrinkage of the cell away from the surrounding neuropil, or of the swelling of astrocyte processes. Granules and rings that stain darkly with haematoxylin are sometimes seen on the surface of nerve cells as a secondary ischaemic cell change. Such cells are frequently placed in a separate category - 'ischaemic cell change

with incrustations'.

Homogenising cell change (HCC). In HCC the cell body becomes pale, opalescent and eosinphilic. The cytoplasm may be unstained with anilin dyes or appear yellowish and more highly refractile than normal. Alternatively, the cytoplasm may appear foamy or granular. The nuclear membrane disappears and the nuclear chromatin becomes clumped around the nucleolus; this stains less deeply than normal with basic anilin dyes and haematoxylin. Purkinje cells undergoing HCC are often surrounded by satellite cells or there may be neuronophagy of the dendrites by microglia. Homogenising cell change appears as a later reaction to ischaemia and/or hypoxia than ICC or ACS, but the nuclear changes are so severe that recovery from HCC seems unlikely.

A fourth nerve cell change has been added to this list by Brown and Brierley (1968). This is "microvacuolation", in which numerous small vacuoles appear in a basophilic cytoplasm. Microvacuolation was the earliest detectable nerve cell change in rat brains following acute, severe ischaemia and/or anoxia.

Synopsis of histological results

The histological results are listed in Tables C25 to C30 and illustrated in Figs. C46 to C76. The method of quantifying nerve cell changes was taken from Brown and Brierley (1968). By this method, the number of nerve cells affected in each of the regions examined is classified as 0 = none, 1 = a few, 2 = several or 3 = many. The hypophysis is not included in the tables because no

cell damage was observed in either the neurohypophysis or adenohypophysis in any of the experiments. Ischaemic cell changes with incrustations were found only in experiment 108 and are not classified separately.

No nerve cell changes were found in any of the 4 control dogs or in the dog maintained under anaesthesia for 3 hours. In the brains of experimental animals, nerve cell changes were of two types - diffuse and focal. Where nerve cells undergoing pathological changes were randomly distributed over a wide area, they were categorised as diffuse cell changes. Focal lesions were small and well defined. They were either oval or wedge-shaped and the widest dimension never exceeded 4 mm. Usually all of the cells and processes within the lesion exhibited pathological changes, but the tissue just beyond the boundary of the lesion was normal.

Diffuse cell changes can be further classified as ischaemic cell change (ICC) and acute cell swelling (ACS). Microvacuolation and homogenising cell change were not found as diffuse changes.

Ischaemic nerve cell changes were found immediately after 2 or 3 hours of non-pulsatile perfusion, but not after pulsatile perfusions of the same durations (Figs. C55 to C57; Tables C25 and C26). They were also found after 1-hour non-pulsatile perfusions with short recovery periods. The severity of ICC varied from mild shrinkage of the perikaryon to marked shrinkage of both the perikaryon and nucleus and corkscrew deformation of the cell processes. Many of

the capillaries in regions of ICC were collapsed and had developed prominent perivascular spaces (Fig. C55).

Acute cell swelling was observed after both recovery and non-recovery experiments (Tables C27 and C28). Except for dog 108 in which ACS was found to have affected some large pyramidal cells of the cerebral cortex, all cases of ACS concerned the cerebellar Purkinje cells (Figs. C57 and C58). Purkinje cells undergoing ACS were found immediately after one 2-hour and two 3-hour non-pulsatile perfusions. In the recovery series, only animals that had received non-pulsatile perfusions were affected by ACS. Two of these were short-term survivors, the other animal survived for 1 week.

Purkinje cells undergoing ACS had grossly swollen perikarya due to the formation of a wide peripheral clear space. Care was taken not to confuse this intracellular space with the space that commonly forms around nerve cells undergoing ICC. It was usual to find focal regions of rarefied neuropil in the molecular layer of the cerebellum close to swollen Purkinje cells. These regions appeared to be formed by swollen Purkinje cell dendrites and possibly swollen granule cell T-fibres, climbing fibre terminals and Bergmann fibre processes. Regions of rarefied neuropil, 10-50μ in diameter, were occasionally seen in the cerebral cortex but the nearby nerve cell perikarya appeared to be normal.

Although ICC and ACS were not prevalent in the brains of

recovery animals a generalised reaction was present in the form of laminar astrocyte proliferation (Fig. C59), vascular cuffing with lymphocytes and polymorphonuclear leucocytes (polymorphs), and phagocytes exhibiting Maltese cross anisotropy in the leptomeninges (Fig. C60).

Isolated focal lesions were found in the brains of 3 non-recovery animals and in all of the recovery animals except number 156 (Figs. C77, C78 and C79. Tables C29 and C30). In the non-recovery animals cellular changes were minimal. The lesions consisted of regions of pallor and swollen cell processes (Fig. C61). Nerve cells exhibiting pronounced ICC were present in these lesions along with nerve cells that stained very palely and appeared to be deficient in cytoplasmic organelles and nuclear chromatin (Fig. C62). Pallor and rarefied neuropil were also found in the brains of recovery animals but in some lesions, nerve cell loss was complete and the region had become occupied by numerous lipid phagocytes and fibroblasts (Figs. C63 and C64). Swollen astrocytes were located near the margins of these lesions (Fig. C65).

Experiment reports

In the following sub-section, pertinent aspects of the perfusion procedure, recovery, post-mortem findings and histological details are described.

Controls

Experiments 78, 100, 104 and 107.

On its removal from the skull, each of the four control brains appeared white and firm. Gross sectioning revealed uniform fixation throughout the brain and no pink or soft regions. A little blood remained in the terminal branches of the cerebral arteries of dogs 78 and 107, and in the sagittal and posterior transverse sinuses of all four brains. All other vessels appeared to be devoid of blood on macroscopic inspection.

Despite the persistence of some arterial blood in experiments 78 and 107, only a few cerebral capillaries and veins contained erythrocytes in histological sections. No fixation artefacts were recognised in any region of any of the control brains. Details of the normal histological appearance of nerve cells and possible artefacts are provided in Appendix 2. Normal nerve cells are illustrated in Figs. C46 to C51.

In all of the dog brains examined, groups of small, round nuclei were found in the septal area, anterior putamen and caudate nucleus. They were particularly numerous beneath the ependymal cells lining the lateral ventricles (Fig. C52) and occasionally appeared to be erupting into the lateral ventricles (Fig. C53). Neuropophagy of apparently healthy neurons could usually be found in the head of the caudate nucleus (Fig. C54). These groups of nuclei are considered to have no pathological significance. It is possible that they represent stages in the normal dispersal of neuroglia from their sites of origin in the ependyma and may be related to the medulloblasts of immature human brain.

Experiment 118.

Anaesthesia was induced with 0.25g sodium thiopentone (Intraval) and maintained for 3 hours with a gas mixture consisting of oxygen 2 l/min., nitrous oxide 0.5 l/min. and halothane 0.5-1.0%. The surgical procedures for perfusion-fixation were performed as usual except that placement of the purse-string sutures and incision of the apex of the left ventricle were postponed until the end of the period of anaesthesia. This experiment was performed to provide a further control brain for comparison with the brains of dogs subjected to 3 hours of extracorporeal circulation.

The only signs of residual blood were found in the sagittal and posterior transverse sinuses. There were no histological differences between this brain and the other control brains.

Non-recovery experiments

1 hour perfusions

Pulsatile experiment number 83

This experiment was one of the earliest attempts to obtain an extracorporeal circulation with the Morton-Keele pulsatile pump. The blood circulation was briefly arrested when the spool valve assembly became disconnected. DeBakey roller pumps had been arranged in parallel with the pulsatile pump to facilitate cannulation and they were quickly employed to re-establish blood flow while the spool valve was reassembled and pulsatile perfusion was continued. The total duration of this incident was only 5 minutes but the transient blood stasis may have had more prolonged effects upon cerebral blood

circulation.

The brain was feintly pink in the frontal and parietal regions of the cerebral cortex on the right side, though the few cells undergoing ICC were found in the occipital cortex. Although not sufficiently severe to be classified as ICC, many cortical neurons were slightly shrunken with narrow extracellular spaces and dark-staining cytoplasm. Some capillaries and veins in the cerebral cortex contained residual blood cells.

Non-pulsatile experiment 123

During the first 30 minutes of perfusing dog 123, the AP increased steadily. Arterial blood carbon dioxide tension fell to 16mm Hg and was over-compensated by the administration of 8.5% carbon dioxide (CO_2). The $P_{a_{CO_2}}$ then increased to 105mm Hg but returned to normal soon after discontinuing the CO_2 ventilation.

No neuropathological changes were found in the brain.

2 hour perfusions

Pulsatile experiment 124

The perfusion of dog 124 was uneventful and well controlled. The AP fell to 15mm Hg at the end of perfusion but fixation was satisfactory and no macroscopical or microscopical abnormalities were found in the brain.

Non-pulsatile experiment 120

A 3-hour perfusion was intended for dog 120 but blood-stained

bronchial fluid formed after 1.5 hours and the perfusion was terminated after 2 hours because of the poor prognosis.

The cisterna magna had been catheterised for CSFP monitoring and like all other brains in which cisternal catheterisation was performed, the subarachnoid space contained a large quantity of blood.

Cortical neurons were generally more darkly stained with CFV than normal and both extracellular and perivascular spaces were widespread in the cerebral cortex and cerebellum (Fig. C55). Many capillaries in the cerebral cortex were distended with erythrocytes but other capillaries nearby had collapsed and were empty. Acute cell swelling was detected in the Purkinje cells.

The pons and medulla were mechanically damaged during cisternal catheterisation and this was thought to have caused the severe ICC found in the giant celled reticular nucleus, dorsal vagal nucleus, inferior olivary nucleus and interpeduncular nucleus. These changes are not recorded in Table C25. Blood cells that had entered the subarachnoid space and fourth ventricle, presumably from damaged blood vessels, contained numerous polymorphs and phagocytes exhibiting Maltese cross anisotropy.

Four small focal lesions were present in the cerebral cortex. Two of these were in the grey matter of the right frontal lobe. The largest of these two lesions was approximately 1mm x 2mm in the plane of the section. Two other lesions were located in the grey

matter of the anterior parietal and parasagittal cortex respectively. Each of the four lesions consisted of a region of pale-staining rarefied neuropil containing nerve cells undergoing ICC and some nerve cells that stained very palely with CFV and haematoxylin. The pale-staining cells appeared to be deficient in both cytoplasmic organelles and nuclear chromatin.

3-hour perfusions

Pulsatile experiments 92, 93, 111 and 127

In the 3-hour pulsatile series, experiments 92 and 127 were uneventful. Two short spells of non-pulsatile circulation were required when the pulsatile pump failed in experiment 93. A CSFP catheter had been placed in the cisterna magna. The CSFP rose sharply to 45mm Hg coincidently with the first change to non-pulsatile perfusion. Venous pressure increased to 30cm H_2O at the same time. Blood-stained bronchial fluid was formed during the perfusion of dog 111. The arterial blood became desaturated after the first 30 minutes of perfusion and large volumes of blood-stained fluid were regularly sucked from the trachea thereafter.

The only pathological nerve cell changes seen in these four brains were one focal region of pallor and rarefied neuropil in the cerebral cortex of dog 92 and three similar regions in dog 93. Nerve cells undergoing ICC and swollen, pale-staining nerve cells were present in the occipital lesion of dog 92 (Fig. C66). Astrocytes

and oligodendrocytes had dark-staining nuclei and swollen pale-staining perikarya. Large numbers of polymorphs were present among the cells and in the sinusoids of the adenohypophysis of this animal (Fig. C67). Two of the regions of focal pallor found in the brain of dog 93 were located in the right occipital cortex and the third one was located in the parasagittal cerebral cortex on the left side. All three lesions were located in the grey matter. There were no signs of ICC in these three regions but some nerve cells stained palely with CFV and may have been slightly swollen.

No signs of nerve cell damage were seen in the brain of dog 111 but CFV-staining debris was present in the great vein of Galen (Fig. C68).

Non-pulsatile experiments 84, 85, 91, 94, 112, 122 and 149

The pulse amplitude reached its maximum value for non-pulsatile perfusions in experiment 84. Probably due to the small size of the dog (body weight 7.25 Kg) and the use of wide tubing (9mm internal diameter instead of 6mm) abdominal aorta pulse pressures up to 30mm Hg were recorded though the pulse wave form remained the same as in other non-pulsatile perfusions. In this experiment, the arterial blood became slightly desaturated during perfusion. In experiment 85, intubation difficulties may have caused some hypoxia. Blood loss totalled 1500 ml during extracorporeal circulation but this was replaced by transfusion. Blood-stained fluid was formed in the trachea after

the first 1 hour of perfusion in experiment 112. The other four experiments in the 3-hour non-pulsatile group were uneventful.

Perfusion-fixation tended to be less successful after non-pulsatile than after pulsatile experiments judged by the macroscopic appearance of the brain (Table C48). An attempt was made to improve the fixation in experiment 149 by using 5 l of fixative instead of 2 l. Perfusion-fixation was judged to be excellent in this experiment.

Ischaemic cell changes were found in all 7 animals in the 3-hour non-pulsatile group. Dog 149 had fewer damaged cells than 4 other dogs in the group, but more than the other 2 dogs. Ischaemic cell changes were found in the cerebral cortex in 6 animals and in the cerebellar Purkinje cells in 5 animals. The basal ganglia, thalamus and lower mid-brain nuclei were less frequently affected. The boundary zones of the major cerebral arteries were affected by ICC more often than other regions of the cerebral cortex. Pyramidal nerve cells were affected more frequently than cortical nerve cells and, in experiments 112 and 149, layers 3 and 5 were selectively damaged.

In regions of ICC, the capillaries were often collapsed and both perivascular and extracellular spaces were conspicuous.

Acute cell swelling of Purkinje cells was found in 2 dogs. Rarefied neuropil was found nearby.

A haemorrhage occupied much of the adenohypophysis and arachnoid space of dog 85 but the neurohypophysis was spared (Fig. C69).

Recovery experiments

Pulsatile experiments 105 and 156

Both of these dogs made rapid recoveries after perfusion. They were able to breathe unaided after 1 hour of recovery and could walk a few faltering steps by 6 hours after the end of perfusion. Dog 105 lost 1800 ml of blood during perfusion mainly from a leaking aortic suture line. An equal volume of blood was transfused intravenously. Both dogs made satisfactory progress during the 1 week recovery periods and no neurological changes were apparent.

When the thorax was opened for perfusion-fixation, repair tissue was seen in both of the heart atria and the aorta was thickened at the site of cannulation. A large thrombus occluded the remaining part of the right atrial appendage of dog 105. Both brains were well fixed and the only macroscopical abnormality was the slightly enlarged lateral ventricles of dog 156.

No diffuse cell changes were evident in sections of the brains but there were three focal lesions in the brain of dog 105 (Fig. C79 and Table C30). The largest of these lesions was located in the corpus callosum. The myelinated fibres had completely disorganized

so that only granules and globules remained to be stained with LFB. Capillary endothelial cells were greatly thickened and the few persisting oligodendrocytes were swollen. Many erythrocytes had transuded into the lesion. Numerous distended lipid phagocytes had invaded the region and most of these had contents that stained with LFB. A similar smaller lesion was located in the pulvinar nucleus of the right thalamus. A third lesion was found in the right frontal lobe of the cerebral cortex. There were signs of nerve cell loss in this lesion. The neuropil was rarefied and pale-staining with CFV. Erythrocytes were present in the capillaries and some cells had exuded into the surrounding brain tissue. No lipid phagocytes were present but microglial cells were increased in numbers. Plump astrocytes could be seen near the margins of focal lesions.

In contrast the brain of dog 156 was entirely normal.

Non-pulsatile experiments 86, 108 and 119

These three experiments were terminated by perfusion-fixation a few hours after by-pass because of poor prognoses.

Dog 86 received 2 hours of non-pulsatile blood flow with flow rates varying between 30 and 75 ml/Kg body weight/min. The perfusion was uneventful but post-operative recovery was slow. Although the dog attempted to breathe unaided, respiratory failure soon occurred when the endotracheal tube was disconnected from the ventilator.

Several such attempts were made between 6 and 7 hours after perfusion, but on each occasion the heart action quickened and AP increased to about 180mm Hg. The AP decreased to 130mm Hg when artificial ventilation was resumed. At this stage, corneal reflexes were present and the pupils were contracted. An attempt was made to improve the respiratory condition by slow intravenous administration of an analeptic (3 ml vanillic acid diethylamide, Vandid). This produced a remarkable temporary rise in AP from 130mm Hg to 240mm Hg accompanied by bilateral proptosis. The AP fluctuations and eye reactions were recurrent during the next 30 minutes but eventually the abnormal condition subsided and the animal returned to its pre-analeptic treatment state. The thorax was then re-opened and perfusion-fixation performed.

The lateral ventricles of the brain were slightly enlarged but no other abnormalities were apparent at post-mortem. Histological examination revealed that many cerebral blood vessels were congested with blood cells. Intravascular polymorphs and lymphocytes were numerous. A focal region of rarefied, pale-staining neuropil, nerve cells undergoing ICC and cell remnants was located in the dorsal thalamus on the right side (Fig. 78). Two capillaries close to this lesion contained Nissl-staining debris. Fibrin was present in a very recent thrombus in a small artery in the left parasagittal cerebral cortex, but the nearby pyramidal and cortical cells appeared to be normal. A petechial haemorrhage had formed on the boundary

between the molecular and granular layers of the cerebellum.

There were no problems during the perfusion of dog 108 but during weaning off the pumps, blood-stained fluid was exuded from the trachea. Nine attempts were made to restore normal breathing by disconnecting the endotracheal tube during the 7.5 post-operative hours. Respiratory movements ceased within 1 minute of disconnecting the endotracheal tube on each occasion. Neurological signs including pupil size, shivering, and pain and corneal reflexes were good, but the tracheal axudate continued. In the last attempt to establish normal breathing, the pupils became dilated and did not constrict when artificial ventilation was restored. The thorax was re-opened and perfusion-fixation was performed.

Convincing signs of cerebral oedema were observed at the post-mortem examination. The cerebral gyri were flattened and the sulci were narrowed (Fig. C70). The lateral ventricles were markedly compressed. The cerebellum did not appear to be swollen.

All of the regions of the cerebral cortex that were examined histologically showed marked ischaemic cell changes (Fig. C56). Layer 1 of the cerebral cortex was generally widened and had elongated clear spaces that appeared to be formed by swollen dendrites and astrocyte processes (Fig. C71). It was difficult to find a normal nerve cell in the cerebral cortex. Most of them had well-developed ICC including corkscrew dendrites. Basophilic granules were apparent on the cell membranes of many ischaemic neurons. Several of the

large pyramidal neurons including some Betz cells were swollen and their cytoplasm appeared finely granular (Fig. C72).

Focal regions of pallor and rarefied neuropil were found in the right parasagittal and frontal cerebral cortex. Nerve cells undergoing ICC and pale-staining nerve cells were present in both of these lesions. Nerve cell shrinkage and the collapse of capillary endothelial cells had produced abnormal extracellular and perivascular spaces. Some capillaries contained erythrocytes. There were signs of cell loss in the frontal lesion. In a third similar lesion in the upper mid-brain, several pairs of astrocytes were found. Three very small regions of rarefied neuropil were located in the basal cerebellar granular layer. A few cells with pyknotic nuclei and swollen, apparently empty cell bodies were present in each of these lesions. These cells were probably abnormal oligodendrocytes but they could have been astrocytes or granule cells.

Dog 119 survived only 3 hours after by-pass. At the start of perfusion the AP had fallen to 40mm Hg and it decreased to 10mm Hg during perfusion. Only 50% of the estimated blood flow was attainable because of poor venous returns. Blood loss was considerable during the recovery period (1 L.) but the dog made a rapid recovery to the stage of unaided breathing. However, respirations ceased after 3 hours of recovery. The dog was re-intubated and artificially ventilated but cardiac arrest occurred during preparation for perfusion-fixation.

The brain was macroscopically normal and microscopically diffuse cell changes were detected only in cerebellar Purkinje cells. Many of these cells were acutely swollen and a few were undergoing ICC. Astrocyte hyperplasia was extensive in the parietal cerebral cortex. In places, the astrocytes formed an additional cell layer parallel with the cortical surface and dividing layer 1 into two parts (Fig. C59). In the parasagittal and temporal cerebral cortex, capillary endothelium appeared to be thickened and nerve cell detritus could be seen in a few veins.

Endothelial thickening, capillary and venous congestion and staining pallor were evident in bilateral focal lesions of the commissural tract (Fig. C73).

Non-pulsatile experiments 98, 117 and 144

These dogs survived for 1 week after non-pulsatile perfusions.

The perfusion and early recovery of dog 98 were uneventful. During the 1 week recovery period, the dog maintained a fixed and vacant stare for most of the time, but it responded to calling and its gait and appetite were good.

One week after perfusion, pleural and pericardial effusions were moderate and tissues of the wall of the thorax appeared to be oedematous.

After perfusion-fixation the brain looked normal and microscopically the nerve cells were generally undamaged. However, a

focal lesion was present in the right parasagittal cerebral cortex (Figs. C63 and C79). The lesion was wedge-shaped with its broad base in layer 6 and its narrow face in layer 1. Nerve cell loss was complete, the whole region being occupied by cell remnants, lipid phagocytes, microglia and fibroblasts. Capillary endothelium was greatly thickened. A few swollen nerve cells and plump astrocytes were present near the margin of the lesion (Fig. C65). Myelin sheaths in the subjacent white matter were swollen and fat globules were present among the fibres (Fig. C74). A small artery in the leptomeninges immediately overlying the lesion contained an early adherent thrombus consisting of blood cells and fibrin. Numerous erythrocytes, polymorphs, lymphocytes and lipid phagocytes had transuded into the subarachnoid space close to the thrombosed artery (Fig. C64). A non-adherent thrombus occluded an artery in the sagittal sulcus but no nerve cell damage was evident at the level of sectioning. The only changes detected in the cerebellum were occasional swollen transverse-fibres in the molecular layer.

The uneventful perfusion of dog 117 was followed by a rapid recovery. The dog was extubated 3.5 hours after perfusion and was lively and trying to stand shortly after this. After perfusion-fixation, the brain appeared to be normal and no diffuse nerve cell damage was evident in histological sections. However, Maltese cross phagocytes were more numerous than normal in the leptomeninges particularly ventral to the cerebellum. Although no generlised

nerve cell changes were detected, many blood vessels contained large numbers of polymorphs and leucocytes. A single haemorrhagic lesion was located in the left occipital lobe (Fig. C75). Blood cells and Maltese cross phagocytes were present in this region but nerve cell changes were minimal, being restricted to mild hyperchromasia.

Freshly-drawn homologous blood was used to prime the circuit for experiment 144. No blood-stained fluid was exuded from the trachea though approximately 10 ml was removed by suction 1 hour after perfusion. Extensive atelectasis of the lungs was present at the time of chest closure. The rate of recovery was satisfactory though not rapid. The dog was able to sit upright with support by 5 hours after completing the perfusion. Lethargy was the only neurological sign noted during the recovery period.

After perfusion-fixation, the brain appeared to be normal. No generalised nerve cell changes were seen in histological sections but there was a small focal lesion in the superficial white matter of the left parasagittal cerebral cortex (Fig. C76). The lesion consisted of round spaces among the myelinated fibres. Some of these spaces contained fat globules. The few nerve cells located in this region were severely degenerated. The cell bodies of oligodendrocytes were swollen and both astrocyte and microglial nuclei were hyperplastic and hypertrophied. Erythrocytes were present in the capillaries which had prominent perivascular spaces.

Early organizing thrombi were apparent in two small leptomeningeal arteries overlying the left parietal and right dorsal frontal cerebral cortex. No nerve cell changes were associated with these thrombi.

Discussion

The validity of the histology results

A survey of the possible causes of brain damage following the use of extracorporeal circulation for open-heart surgery was made in Section B of this thesis. Several of those possible causes are not relevant to this present discussion. For example, inadequate cerebral perfusion in the pre-operative period due to cardiovascular pathology may be assumed not to have occurred because the dogs were apparently healthy; pre-operative stress and the psychological pressures of post-operative readjustments to life do not apply to experimental animals, nor are they likely to cause structural cell changes; gas bubbles, fat globules and plasma protein denaturation in oxygenators are not consequences of extracorporeal circulation by the Drew technique; and no period of total circulatory arrest was employed.

The occurrence of widespread ICC immediately after 3 hours of non-pulsatile but not after pulsatile perfusions supports the initial hypothesis that tissue perfusion is better during pulsatile than during non-pulsatile extracorporeal circulation. However,

there were other differences between pulsatile and non-pulsatile perfusions. The following factors must be conceded:

1. There was one important difference in technique. For non-pulsatile perfusions the left femoral artery was cannulated and the direction of blood flow to the chest and abdomen was retrograde. These regions were progradely perfused via the proximal aorta during pulsatile perfusions.

2. Intermediate pathological changes were produced in both groups. Brain damage could have been caused by low arterial blood oxygen tensions ($P_{a_{O_2}}$) following lung damage or due to inadequate lung ventilation. Arterial blood oxygen tensions were not measured but there were only three cases of bronchial fluid formation during non-recovery experiments. These occurred during 2-hour non-pulsatile, 3-hour non-pulsatile and 3-hour pulsatile perfusions respectively. It is possible that extensive damage to the lung capillaries resulted in low $P_{a_{O_{2s}}}$ without causing bronchial blood-stained fluid to be formed.

Secondly, blood damage caused by the two pumps was quite different in extent. At the end of 5 hours of <u>in vitro</u> blood pumping, haemolysis caused by the roller pump was 30-times greater than that caused by the pulsatile pump. Although it may be expected that haemolysis will increase blood viscosity, the effects of haemolysis during extracorporeal circulation

upon microcirculatory dynamics are generally reckoned to be unimportant (Clowes, 1960; Galletti and Brecher, 1962).

3. The primary objective of analysing the physiological data in the previous section was to detect any physiological factors that might explain the histological differences between the two groups of animals. The analysis showed that there were differences in AP, PVR and P_{aCO_2} during 3-hour perfusions.

The differences in AP were partly explained as mathematical artefacts but the increase in AP that occurred during non-pulsatile perfusions was thought to be a consequence of other physiological changes that can be directly attributed to the flow system and not to extraneous factors.

The same argument applies to the differences in PVR that occurred during 3-hour perfusions, but P_{aCO_2} differences cannot be discounted in this way. One of the experimental controls was to maintain a constant P_{aCO_2} by appropriate adjustments to the lung ventilation. When P_{aCO_2} became too high, the tidal volume should have been increased and when P_{aCO_2} fell, CO_2 should have been added to the ventilation gas mixture. Obviously this was not done adequately because P_{aCO_2} increased during pulsatile experiments but remained more constant during non-pulsatile experiments of 3 hours duration. The difference between the two groups may have been due to lung damage caused by the rapid pulsatile pump ejection, though this conclusion was not substantiated by observations of bronchial blood-

stained fluid formation. The important point is that vasomotor responses of the cerebral arterioles are dependent upon cerebral perfusion pressure (AP-VP) and $P_{a_{CO_2}}$ (Harper, 1965; Zwetnow, 1968). Cerebral perfusion pressure becomes important only at extreme values of AP and it is $P_{a_{CO_2}}$ that determines the state of dilatation of the cerebral arterioles within the normal range of APs. Cerebral perfusion is increased by dilation of the cerebral arterioles in response to increases in $P_{a_{CO_2}}$. No information is available about the effect of $P_{a_{CO_2}}$ upon arterioles in non-cerebral regions of the brain, and it has been suggested that normal cerebral blood flow autoregulation is abolished during extracorporeal circulation (Ankeney and Viles, 1961). Harper (1965) has shown that cerebral blood flow autoregulation to changes in $P_{a_{CO_2}}$ is abolished when the AP falls below 80mm Hg in hypercapnic dogs. Cerebral blood flow is then determined by cerebral perfusion pressure alone. Fortunately, AP was always below 80mm Hg in our experiments and AP was lower during pulsatile than during non-pulsatile experiments. Nevertheless, the results presented here should be criticised for the failure to control $P_{a_{CO_2}}$.

To summarise, there are four factors that may have contributed to the histopathological differences found in the brains of dogs immediately after pulsatile and non-pulsatile perfusions of 3 hours duration. These are the direction of flow into the systemic circulation, haemolysis, $P_{a_{CO_2}}$ and the presence or absence of arterial pulsations.

A more fundamental problem is to verify that the nerve cell changes described above are genuine and that they are not fixation artefacts. Of course, great care was taken to avoid fixation artefacts and no artefacts could be detected in control brains or in the brains of dogs that had been subjected to 1 hour of non-pulsatile or 1-3 hours of pulsatile perfusion. However, collapsed capillaries were often present in regions of ICC after 3 hours of non-pulsatile perfusion. Therefore it may be that the true capillaries collapsed during non-pulsatile perfusions and that thereafter the brain tissue dependent upon these capillaries was inadequately perfused. It is also possible that during perfusion-fixation, the fixative was unable to pass through collapsed capillaries and that the nerve cell changes observed in these regions were partly or wholly due to post-mortem autolysis.

Early ICC is difficult or impossible to distinguish from cell changes that result from inadequate perfusion-fixation because the principal element in both mechanisms is a deficiency of oxygen supply to the tissue. The problem is sufficiently fundamental to require a reappraisal of the term 'fixation artefact'. When rigidly defined 'artefact' means "a product of human art and workmanship" (Oxford concise dictionary, 4th edition). It follows that histology is a study of the artefacts resulting from fixation, processing, sectioning and staining of tissues. The valuable distinction to make is between changes from the accepted histological normal that result from

pathological changes of any kind - structural, biochemical or physiological - and those that are the products of inadequacies of histological technique. In the experiments described here, collapsed capillaries were the primary effects of non-pulsatile flows. Nerve cell changes were a second indication of capillary collapse. It can be assumed that nerve cells are injured by ischaemia due to the inadequate capillary perfusion and the only question remaining is whether the nerve cell changes seen in histological sections represent this injury. If the nerve cell changes were induced at the time of perfusion-fixation, it may be expected that the phenomenon would be 'all-or-none'. Nerve cells would be either adequately or inadequately fixed. On the other hand, if the cell changes were the genuine results of non-pulsatile hypoxia/ischaemia there should be a gradation of changes correlating with the duration of extracorporeal circulation; after short perfusions there should be fewer and more subtle changes than after longer perfusions. For this discrimination an evaluation of the quality of nerve cell changes is as important as quantitation. A third possibility is that the final histological result was due to a combination of pathological and artefactual cell changes resulting from capillary collapse.

The next five sections of this thesis may be regarded as complementary attempts to resolve this very refractory problem as well as presentations of additional results of intrinsic value. Details of perfusion-fixation are discussed to determine whether or not the nerve

cell changes can be related to differences in the performance of perfusion-fixation. A comparison is sought between the histology of brains after perfusion-fixation and after immersion-fixation, including the brains of two patients who died after open-heart operations. The development of cell changes is examined by histochemistry and electron-microscopy. Cell counts are presented that disclose changes in the cell populations of the cerebral cortex. The possible causes of focal lesions are considered in later sections.

THE EVOLUTION OF DIFFUSE NERVE CELL CHANGES FOLLOWING EXTRACORPOREAL CIRCULATION

Introduction

The histological results presented in the previous section were based upon the examination of sections stained by routine histological techniques (H-E, H-VG, CFV, LFB-CFV and PTAH). Confirmation of these results was sought by staining sections by the H-PAS technique for 1:2 glycol groups and their derivatives.

Materials and Methods

Paraffin wax sections 7-8μ thick were prepared from all of the brains and stained as follows:-

1. Dewax and take sections through absolute and 95% alcohols to distilled water.
2. Oxidise in 0.5% aqueous periodic acid for 5 minutes.
3. Rinse in distilled water.
4. Schiff's reagent (G.T. Gurr LTD.) in a dark place for 15 minutes.
5. Three 2-minute changes of freshly prepared sulphurous acid.
6. Wash in running tap water for 20-30 minutes.
7. Rinse in distilled water.
8. Mayer's haemalum for 3 minutes.
9. Blue the haemalum in running tap water for 20 minutes.
10. Dehydrate, clear and mount in Canada balsam.

Results

Typical results of H-PAS staining are illustrated in Figs. C80 to C89. The nuclei of normal nerve cells were PAS-negative but the cytoplasm contained PAS-positive granules. These granules were usually disseminated throughout the cytoplasm, but dense clumps of PAS-positive granules were sometimes found in the trigeminal nerve nucleus, and in Purkinje and Betz cells (Figs. C80 and C81). A few small blood vessels of all types had PAS-positive granules in their walls. The neuropil was diffusely but mildly PAS-positive.

Purkinje cells undergoing ACS after extracorporeal circulation retained their PAS-positive granules in the perikarya, and additional granules were sometimes seen in distal regions of the dendrites (Figs. C84 and C85). In contrast, nerve cells undergoing ICC had very few or, more usually, no PAS-positive granules but the cytoplasm of perikarya and dendrites was diffusely and homogeneously PAS-positive (Fig. C86). In cells exhibiting severe ICC the pyknotic nuclei were also PAS-positive (Fig. C87).

The H-PAS staining technique has revealed changes in the neuropil structure that were not evident in sections stained by standard techniques. Fig. C82 shows an apparently healthy Purkinje cell containing a normal distribution of cytoplasmic PAS-positive granules. However, with this technique it is possible to detect a mild rarefaction and loss of the PAS-positivity of the adjacent neuropil. Fig. C83 shows a slightly more advanced stage of neuropil rarefaction and early

signs of the formation of an extracellular space. A few PAS-positive granules are present in the cytoplasm but the diffuse PAS reaction is slightly more intense than normal. A collapsed capillary can be seen close to the Purkinje cell. Patches of rarefied neuropil approximately 50μ in diameter were frequently seen in the cerebellar cortex of experimental animals, particularly close to Purkinje cells undergoing ACS (Fig. C88).

As shown in Table C31, changes in the PAS reactions of nerve cells were always found immediately following 2 or 3 hours of non-pulsatile and usually following 3 hours of pulsatile extracorporeal circulation. Diffusely PAS-stained nerve cells were more numerous after non-pulsatile than after pulsatile perfusions. Both the numbers of nerve cells and the numbers of regions with altered PAS reactions were higher than the corresponding numbers for ICC based upon standard histological staining techniques.

In the recovery series, changes in the PAS reactions of nerve cells were more numerous in the brains obtained after terminated experiments than in those obtained after 1 week recoveries (Table C32).

A few densely PAS-positive cells were seen in the Virchow-Robin space of veins in control sections. These cells were more numerous in the brains of experimental animals and in the non-pulsatile series, they were also found in pericapillary spaces (Fig. C89).

Discussion

It is possible that PAS-positive material is concerned in an early sub-cellular response to hypoxia/ischaemia caused by extra-corporeal circulation. A possible sequence of changes may be that shown in Fig. C90 and illustrated in Figs. C80 to C87. The hypothesis is that rarefaction and loss of PAS-positivity of the neuropil precedes changes in the nerve cell perikarya in the evolution of ACS or ICC. The initial perikaryal reaction may be one of shrinkage or swelling of the cytoplasm and nucleus. If the cell shrinks, PAS-positive granules are replaced by a mild, diffuse PAS reaction of the cell cytoplasm. If the cell swells, the PAS-positive granules are not lost and they may be dispersed to more distal regions of the dendrites. However, cell swelling may be converted to cell shrinkage with the associated loss of PAS-positive granules. Any of these changes may be reversed by the restoration of normal blood circulation or the damaged cells may disappear.

No attempt has been made to identify the PAS-positive material in nerve cells, but the evidence that changes in PAS reactions are more extensive than ICC suggests that a sub-cellular biochemical change precedes ICC in the degenerative sequence. The findings that a loss of PAS-positive material and structural changes in the neuropil precede nerve cell changes, that apparently healthy nerve cells had altered PAS reactions, and that changes in the PAS reactions were

found in regions where no capillaries were collapsed indicate that the pathological changes observed in nerve cells after extracorporeal circulation are graded and not 'all-or-none'. Further comments are made in the 'general discussion'.

HISTOLOGY OF BRAINS FIXED BY IMMERSION TECHNIQUES

Introduction

Three brains were obtained following whole-body perfusions by the Drew (1961) technique after which the subjects died. These brains were fixed by immersion for comparison with perfuse-fixed material. By including one experimental dog brain and two brains of patients who had died after open-heart operations, it was intended to facilitate the extrapolation from experimental dog perfusions to clinical experience.

The dog brain.

Experiment 154.

Materials and Methods

A 10.9 Kg beagle dog was perfused for 1 hour at 31°C with pulsatile blood flow. The AP remained around 26mm Hg throughout the perfusion but blood flow rates were maintained at about 700 ml/min. (63.1 ml/Kg/min.). The post-operative recovery was rapid and blood loss in the recovery period was less than 50 ml. The animal was able to stand 6 hours after terminating the perfusion and all of the neurological signs were good. However, the dog died during the night at a time when it was not under observation. The recovery period was between 10 and 12 hours. Eight to ten hours later, the brain was removed and fixed by suspension in Cammermeyer's Susa

for 1 week, after which it was trimmed to give the 13 tissue blocks listed in Appendix 2 and processed as usual.

Post-mortem observations

Three focal haemorrhages were present in the lungs and there was some atelectasis of the left lobes. The intestines were anaemic but splanchnic veins were congested. The brain was swollen. The gyri were flattened, the sulci were narrowed and the ventricles were compressed.

Microscopical observations of the brain

Ischaemic and acute nerve cell changes were found in the pyramidal nerve cells of layers 3 and 5 in all of the regions of the cerebral cortex that were examined (Fig. C91). Ischaemic cell changes were also numerous in the hippocampus, olfactory tract, amygdaloid nuclei, inferior olivary nucleus and Purkinje cells of the cerebellum. Widened extracellular and perivascular spaces were ubiquitous and many capillary walls appeared to be collapsed.

A small region of pallor and cell loss was located in the left occipital cortex. There was no evidence of cellular reaction in this region or nearby.

The human brains.

Materials and Methods

The brain of Mr. W. was fixed for 3 months and that of Mr. S. for 6 months by suspension in 4% formaldehyde in 0.9% sodium chloride.

The brains were trimmed to give 17 tissue blocks each approximately 5mm thick:-

Cerebral cortex - frontal lobes (2 blocks)
- occipital lobes (2 blocks)
- temporal lobes including Ammon's horn (2 blocks)
- central cortex including sensory and motor regions (2 blocks)

Thalamus (2 blocks)

Caudate nucleus (2 blocks)

Upper mid-brain

Lower mid-brain

Pons

Medulla

Cerebellum including dentate nucleus

The 17 tissue blocks were post-fixed by immersion in 4% formaldehyde in 0.9% sodium chloride for 2 hours and processed on an automatic tissue processer:-

1. Four 8-hourly changes of propan-2-ol with increasing alcohol content from 95% to 100%.
2. Absolute ethanol: diethyl ether (1:1) for 9 hours.
3. 1% celloidin at 32°C for 7 hours.
4. 2% celloidin at 32°C for 15 hours.
5. Absolute ethanol:diethyl ether (1:1) for 10 minutes.
6. Two 8-hourly changes of chloroform.

7. Two 5-hourly changes of paraffin wax, melting point 55°C (Paramat, G.T. Gurr Ltd.).

At this stage the tissue carrier was transferred to a third bath of paraffin wax in an embedding oven and evacuated to 600mm Hg for 4 hours, followed by a further change of wax and evacuation for 2 hours before final embedding. Sections were cut and stained as previously described.

Pathology reports

1. Mr. W.

Mr. W. was a 64 years old man who had a 6 months history of retrosternal pain and dyspnoea upon mild exertion. A heavily calcified aortic value, post-stenotic dilation of the aorta and left ventricular hypertrophy were found during surgery. Non-pulsatile extracorporeal circulation was established and the aortic valve was replaced by a prosthesis during 80 minutes of circulatory arrest at 10°C. Post-operatively, repeated episodes of atrial fibrillation and asystole eventually resulted in an irreversible cardiac arrest 2 days after surgery.

Post-mortem observations

Subendocardial haemorrhages were present in the heart and there was an atheromatous plaque immediately above the aortic valve. The lungs were congested and there was marked emphysema of both upper lobes. The brain weighed 1310g and was macroscopically normal.

Microscopical observations of the brain

Nerve cells in the cerebral cortex were seen to contain a yellow pigment that was probably lipofuscin (Fig. C92). Most nerve cells in the cerebral cortex were undergoing non-specific changes probably of long duration. Many blood vessels were congested with polymorphs and lymphocytes (Fig. C93) and several blood vessels showed perivascular cuffing with the same cells. Scattered ACS and ICC of Purkinje cells were the only diffuse cell changes found in the cerebellum (Fig. C94).

There were seven focal lesions in the cerebral cortex and basal ganglia (Fig. C95). Three of these lesions were located in the left temporal lobe of the cerebral cortex and there were two lesions in the left occipital lobe. The other two lesions were located in the left motor cortex and the left caudate nucleus. The lesion in the caudate nucleus was approximately 4mm x 1mm in the plane of the section. It consisted of a region of intense infiltration with polymorphs, lymphocytes and lipid phagocytes. Five of the other six lesions contained large cystic spaces occupied by lipid phagocytes and a few fibroblasts. Thickened capillary endothelium was evident in the lesion located in the motor cortex. Plump astrocytes were present near the margins of the lesions. Nerve cell loss was complete in all of the lesions except the largest one which was located in the left occipital cortex. This lesion consisted of a 1cm x 1cm region of staining pallor with swollen astrocytes and lipid phagocytes near the centre.

A focal mixed coccus and bacillus contamination of nerve cells and blood vessels was found in the right motor cortex (Fig. C96). This may have been a post-mortem phenomenon. Corpora amylacea were widely scattered in the cerebral cortex and they were highly concentrated in layer 1 of the left arterior temporal lobe (Fig. C97).

2. Mr. S.

Mr. S. was a 47 years old man who had suffered from substernal discomfort and recurrent left ventricular failures for 5 months. Syphilitic aortic regurgitation was diagnosed as a result of clinical investigations. Non-pulsatile extracorporeal circulation was established and the aortic valve was replaced by a prosthesis during 42 minutes of circulatory arrest at 10°C. During weaning off the pumps, the left ventricle failed temporarily and blood-stained fluid was exuded from the trachea. The patient recovered consciousness post-operatively, but his condition deteriorated rapidly and a dissociated ECG rhythm and bradycardia were followed by cardiac arrest 7 hours after surgery.

Post-mortem observations

The left ventricle had become enormous. The papillary muscles were necrotic and there were a few subendocardial haemorrhages. The wall of the aorta was markedly thickened and showed linear corrugations of the endothelium. Intimal and subintimal plaques characteristic of syphilitic arteritis were observed. The lungs were grossly congested. The brain weighed 1400g. A recent haemorrhage approximately

1cm x 0.5cm x 0.5cm was detected in the left dorsal occipital cerebral cortex mainly involving the white matter.

Microscopical observations of the brain

Extensive ICC of the cerebral pyramidal cells was the only ischaemic/hypoxic nerve cell change found in the cerebral cortex (Fig. C98). This was more pronounced in layer 3 than in layer 5 and the temporal lobes were affected more than other cerebral regions. Some Purkinje cells were also seen to be undergoing early ICC.

The only foreign cells present in the occipital lobe haemorrhagic region were erythrocytes and a few polymorphs. Blood vessels containing numerous polymorphs and lymphocytes were seen in the thalamus. Isolated groups of corpora amylacea were located in the right olfactory tract, right occipital cortex, subependymal grey matter and cerebellar peduncle.

Discussion

Ischaemic/hypoxic nerve cell changes were found in all three of the brains fixed by immersion techniques. These cell changes were mainly chronic in the brain of Mr. W. but recent cell changes were found in all three brains. However, ICC cannot be diagnosed with any confidence because of the possibility of fixation artefacts. The presence of numerous polymorphs and lymphocytes in some blood vessels of both of the human brains may have been a recent response to cerebral insult.

The focal lesions found in the brain of Mr. W. were too advanced to have been initiated during the open-heart operation and they were probably caused by reductions in cerebral blood flow associated with the cardiovascular defect. The focal lesion found in Mr. S's brain was recent but could have been produced prior to surgery.

These findings confirm the importance of perfusion-fixation in studies of early ischaemic/hypoxic nerve cell changes. The difficulties involved in determining the causes of neurological defects following open-heart surgery are also demonstrated. It is clear that pre-operative factors can complicate neuropathological studies of brain damage resulting from extracorporeal circulation in human patients. However, the results are compatible with those obtained by perfusion-fixation of dog brains and, although possibly due to fixation artefacts, the recognition of early ACS and ICC in these brains tends to support the previous conclusions.

CEREBRAL CELL COUNTS

Introduction

Regions of cell loss and pallor created by localised ischaemia are easily detected in light-microscope sections. Diffuse cell loss produced by generalised ischaemia and/or hypoxia is not so easily recognised because of the very large numbers of cells present in the cerebral cortex. For this purpose, extensive cell counts are required. The production of the Quantimet Image Analysing computer by Metals Research Limited has facilitated cell counting in microscope sections.

Materials and Methods

The Quantimet was designed and constructed for petrological applications, but its biological uses have recently become apparent. The arrangement of the Quantimet and its accessories is shown in Fig. C99. The object image is transmitted by the microscope onto the screen of a television camera. The electrical output from the camera is relayed to a closed circuit television monitor to provide a normal television image of the object, and also to a detector unit. Signals from the camera emanating from the features to be measured are discriminated and selected from the rest of the signal in the detector unit. The detector output, consisting of pulses from the selected features, can be relayed to the monitor to produce a display

of the selected features, and to the computer unit which is programmed to select features for counting on the basis of size and optical density. The measurements are presented on the meter or as a table compiled by an automatic typewriter. Mechanisms are available to prevent single large objects being counted as two smaller objects i.e. the instrument recognises multiple intercepts. These mechanisms become less reliable when counting elongated structures, so Nissl preparations were chosen because these reveal only the most proximal parts of the dendrites and axon hillock.

Sections of the cerebral cortex 15μ thick and stained with CFV were used for cell counts in the frontal (left rectal gyrus) and occipital (right posterior suprasylvian gyrus) regions. Using a Watson Image-Shearing Eye-piece (WISE), the longest (a) and shortest (b) dimensions of objects categorised as neuroglial and endothelial cell nuclei, pyramidal nerve cells and cortical nerve cells were measured on control brain sections. Cell types were differentiated morphologically and 100 cells of each type were selected from the two brain regions. The shortest dimension was measured as the greatest dimension in the plane perpendicular to the longest dimension as shown at the head of Table C33. These measurements are those usually obtained for estimating cell volumes (Shariff, 1953; Bok, 1959).

For cell counts with the Quantimet, the computer was programmed to classify and count cells in the size ranges (cords) 0.5-10μ, 10-22μ and $>22\mu$. The slide was positioned on the rotary stage of

the microscope so that serial counts were made in columns perpendicular to the gyral surface from the pia-arachnoid membranes to the subjacent white matter. This usually included 10-12 fields with dimensions of 169.23μ x 146.15μ (24,732.9645μ^2). The fields were arranged to be just non-overlapping in each column and the columns were 0.8mm apart. Ten columns of cells were scanned on each section, i.e. 100-120 fields per section. However, this number was reduced to approximately 80% by excluding all fields overlapping the boundaries between layers 1 and 2, and between layer 6 and the white matter.

Table C34 is a typical automatically typed record of the Quantimet counts made on one column of fields. The total count includes any granular material present in the section. This is eliminated in the second column of figures by raising the minimum cord setting to 0.5μ. The size range 0.5-10μ is then obtained by deducting the figures in column 3 from those in column 2. The numbers of cells in the remaining two size ranges are calculated by deducting column 4 figures from column 3, and column 4 gives the results for the size range $>22\mu$. The final column of figures provided by the Quantimet is the percentage area covered by cells in the size ranges counted. These figures were not used. The annotations made on Table C34 were made at the time of counting to denote the cell layers included in each field. From the figures included in the rectangle, the mean values were obtained for each

size range and entered in another table such as Table C35, from which the mean values representing the cell counts for the section were calculated.

Finally, the effects of section compression were compensated by multiplying the mean values for each section by a constant k. The constant was derived for each section by the formula:-

$$k = \frac{\text{block length (mm)}}{\text{section length (mm)}}$$

after obtaining measurements of the lengths of the embedded tissue block and of the stained section on the slide. The range of values for k was 1.000 to 1.159 for frontal lobe and 1.018 to 1.152 for occipital lobe sections (i.e. section compression was between 0% and 15.9%).

Limitations of the Quantimet

During preliminary test counts with the Quantimet, certain limitations of use became apparent. Although the microscope could be set to give Koehler illumination of the object, definition of the iris diaphragm using the Bertrand lens was poor and there was no calibration of the substage adjustment. Movements of the substage of as little as 1mm made considerable differences to the threshold setting of illumination. Therefore, it was decided to make a series of tests to determine the repeatability of results and to examine the effects of different threshold settings.

Each day, before making a series of counts, a test slide was

counted to provide a control of counting variables. Results obtained on other sections that day were discounted if the results for the control section differed markedly from those obtained on other days. In the last three attempts to repeat the control counts, unacceptable values were computed and no further results have been obtained. A representative of Metals Research Limited has informed the owners of the instrument (Imperial Chemical Industries Limited) that an uncorrectable deterioration has occurred in the electronic circuitry. Because of this, no counts are available for 5 frontal and 11 occipital lobe sections.

Two further groups of cell counts were made with the Quantimet to determine the effects of slight alterations of the threshold setting. All of these preliminary test counts were made on the same section of the rectal gyrus of dog 120.

Results

(a) Cell dimensions

The longest (a) and shortest (b) dimensions of neuroglial and endothelial cell nuclei, and of cortical and pyramidal cell perikarya are shown in the histograms, Figs. C100 and C101. The relationships between these groups of cells and the size ranges used for counting are shown in Table C33. The errors introduced by cells being sectioned in different planes is small except for pyramidal cells in the size range 10-22μ of the dimension b. However, sections were cut in the same plane as the long axes of the majority of nerve cells, so the

values of a are more appropriate than those of b for relating cell dimensions to cell types. The columns headed %a show the correspondence between cell lengths and cell types, e.g. all of the cells counted in the size range > 22μ are either cortical or pyramidal cells and it is likely that 89.2% of these will be pyramidal cells.

(b) Repeatability of cell counts with the Quantimet

Test count results are shown in Tables C36 to C38 and in Figs. C102 to C104. Table C36 is a list of the results of 11 cell counts made on the same section on 6 different days. Count numbers 1 to 5 were made consecutively on the same day without altering any of the microscope, detector or computer settings.

No reliability can be placed upon the various Quantimet settings for reproducing counting conditions. Count numbers 6 to 11 in Table C36 were made on 6 different days and the Quantimet was prepared for use according to a meticulous schedule on each occasion, but the optimum threshold setting required to give the same display effects varied from 535 to 700. The mean total numbers of cells counted in the grey matter varied between 60.9 and 73.0 cells/field but these values did not correlate with the threshold settings ($b_{yx} \doteq 0$). However, the numbers of cells counted in each size range did correlate with the mean total cell count for the grey matter (r_{xy} in Table C39). Fig. C102 shows positive correlations between the grey matter cell count values for the size range > 22μ and the total cell count ($b_{yx} = 0.470$), and for the size range 10-22μ and the total cell

count (b_{yx} = 0.704), but a negative correlation between the count values for the size range 0.5-10μ and the total cell count (b_{yx} = -0.178).

If the mean values for the 11 cell counts listed in Table C36 are arbitrarily selected as the correct values, the correction factors required to convert any cell count made on the region of the slide examined to the correct values can be derived from Fig. C103. This shows that for mean total cell counts in the range 60.9 to 73.0 cells/field, the correct values are obtained by multiplying as follows: 0.5-10μ (0.975 to 1.035), 10-22μ (1.228 to 0.741) and >22μ (1.310 to 0.702). Thus the ranges of error for these 11 cell counts were +2.5 to -3.5% (0.5-10μ), + 25.9 to -22.8% (10-22μ) and +29.8 to -31.0% (>22μ). The range of error for the mean total grey matter cell count was +11.9 to -8.5%. For cell counts in the size range 0.5-10μ in the white matter, the range of error was +9.2 to -39.7%. Only occasional nerve cells are located in the white matter of the cerebral cortex and these have been recorded but ignored for the purposes of calculation.

When the cell counts for each size range are related to the total cell count according to Fig. C103, the ranges of error are +4.5 to -3.9% (0.5-10μ), +8.8 to -9.0% (10-22μ) and +18.2 to -17.4% (>22μ) for the grey matter. The range of error for the white matter size range 0.5-10μ becomes +115.2 to -36.3%.

Table C39 and Fig. C103 show that the correlation and regression

coefficients relating the numbers of cells in the size range 0.5-10μ in the white matter to the total cell count for the grey matter are approximately equal to those relating the corresponding cell size range in the grey matter to the total cell count for the grey matter, but the signs are reversed (columns 1 and 4, r_{xy} and b_{yx} of Table C39). A possible explanation of these relationships is that when the Quantimet counting parameters are adjusted to give slightly low values, large objects in the field are identified as several smaller objects. Thereby, cells in the size range 10-22μ are transposed to the size range 0.5-10μ. This accounts for the negative correlation between the numbers of cells in the size range 0.5-10μ in the grey matter and the total cell count for the grey matter.

The same effect is not obtained for the size range 0.5-10μ in the white matter because there are very few cells with diameters exceeding 10μ in that region. Since the regression line relating cell numbers in the size range $>22\mu$ to the total cell count is not so steep as that relating cell numbers in the size range 10-22μ to the total cell count, it is also possible that a transposition occurs between these size ranges when the Quantimet counting parameters are adjusted to give slightly low values. Transpositions in the opposite direction (from left to right in Table C36) may occur when the Quantimet counting parameters are adjusted to give slightly high values. This would occur if the Quantimet's efficiency in distinguishing individual cells in a closely set group of small cells is reduced when the Quantimet counting parameters are set to give slightly high values.

Observations of the television display monitor during Quantimet adjustments support this hypothesis. It also accounts for the large differences in variation of results between grey matter and white matter cell counts in the size range 0.5-10μ (d in Table C36).

(c) The effect of threshold variations

Tables C37 and C38 and Fig. C104 show the effect of varying the threshold setting with all other factors constant. Raising the threshold setting increases the total cell count and the values obtained for all size ranges in both the grey and white matter. The differences between the coefficients r_{xy} and b_{yx} for fixed and variable thresholds denote that threshold is not the only factor responsible for the divergence of results in Table C36 (Table C39).

(d) Cell counts on sections of the brains of experimental dogs

(i) The limits of error

For the purpose of deciding whether or not the cerebral cell counts of experimental animals differed significantly from control brain values, the 95% confidence intervals were determined for each cell size range by the formula:

$$\bar{x} \pm 1.96d.$$

These values are shown in Table C40. The mean values and confidence intervals were then adjusted by adding to both values the percentage error determined in sub-section b. The maximum and minimum values thus determined are listed in Table C41. These denote the limits of error for control brain values.

(ii) Cell population changes

Tables C42 to C45 show the results of cell counts made on sections of control dog brains and of the brains of dogs subjected to extracorporeal circulation. Values beyond the limits of error for control brain sections are underlined. Changes in the cerebral cell populations following non-recovery experiments are summarised in Table C46 in which '+' indicates an increase in cell number in excess of the control brain limits of error and '-' indicates a decrease below these limits.

Non-recovery experiments. Although the number of experiments in each group was low, it seems reasonable to conclude that there was a greater tendency for cell population changes to occur after non-pulsatile than after pulsatile perfusions.

All changes in the frontal lobe were increases in cell numbers, but both increases and decreases were found in the occipital lobe. In the cell size range 0.5-10μ of the white matter, only 2 brains had values above the control brain limits of error but 6 brains had increases in the same size range in the grey matter. Except for one dog following 3 hours of anaesthesia alone, the increases in the size range 0.5-10μ were all found in the non-pulsatile group.

The only decreases in cell numbers were found in the grey matter size ranges 10-22μ and >22μ after 3-hour non-pulsatile perfusions.

Recovery experiments. Changes in cell numbers were more frequent in

the frontal than in the occipital lobe. There were no changes in the size range 0.5-10μ of the grey matter of the occipital lobe after one pulsatile or after three non-pulsatile experiments. In the frontal lobe, two increases and one decrease were recorded following non-pulsatile perfusions. The white matter cell count of the frontal lobe was increased after one pulsatile and one non-pulsatile experiments, and decreased in the occipital lobe following two terminated non-pulsatile experiments.

In the cell size ranges 10-22μ and >22μ, increases were computed in the frontal lobe following the only pulsatile and following 4 of the 5 non-pulsatile experiments.

Discussion

Increases in the numbers of cells recorded in any size range can be produced in several ways:-

(a) Cells may infiltrate into the counting areas from other regions, e.g. polymorphs;

(b) Cells within the counting area may multiply by division;

(c) Cells may differentiate in response to various stimuli, e.g. microglia may become lipid phagocytes;

(d) Cells may swell or shrink and thereby be transposed from one size range to another.

It is generally believed that nerve cells in the brain do not multiply by division once they have passed the neuroblast stage (Greenfield and Meyer, 1963). In the sections of dog brains used

for the above cell counts, there was no evidence of the multiplication of nerve cells such as mitotic figures or multinucleation. Therefore, increases in the numbers of cells counted in the size range >22μ cannot have been due to cell multiplication because all of the cells included in this size range are considered to be nerve cells (Table C33). A maximum of 6.9% of the cells included in the size range 10-22μ are considered not to be nerve cells. It is possible that increases in the numbers of cells in this size range were due to the multiplication of cells such as astrocytes and oligodendrocytes but the small size of newly-formed neuroglial cell nuclei makes this explanation unlikely. However, this could explain the increases in cell numbers recorded in the size range 0.5-10μ.

Infiltration of cells such as polymorphs could also explain increases in the size range 0.5-10μ. Although no polymorphs were seen in the regions used for cell counting in any sections of the brains of non-recovery animals, it is possible that other less-easily recognised phagocytic cells such as those originating in the Virchow-Robin space could have migrated into the cerebral cortex (Maxwell and Kruger, 1965). Increasing numbers of microglia are known to follow cerebral injury (Huntington, 1966). These would also be included in the size range 0.5-10μ unless they had differentiated to form lipid phagocytes. Neither swollen microglial nuclei nor lipid phagocytes were seen in any of the regions used for cell counts.

The proliferation of endothelial nuclei would make a further contribution to the size range 0.5-10μ though it is unlikely to account for any increases in cell numbers in the other size ranges.

The numbers of cells in the size range 10-22μ are most likely to be increased by the swelling of smaller cells or by the shrinkage of larger cells. The numbers of cells in the size range $>22\mu$ can only be increased by smaller cells swelling.

The preferred conclusion must be that increases in the numbers of cells counted in the various size ranges were due to both cell swelling and cell multiplication and/or cellular infiltration. These effects were more obvious in the grey matter than in the white matter and more frequent following non-pulsatile than following pulsatile perfusions. The increases in cell numbers found in experiment 118 in which thoracotomy was performed under anaesthesia but in which extracorporeal circulation was not employed are surprising but further experiments are needed before drawing any definite conclusions. This point will be reconsidered in the general discussion.

Decreases in cell numbers were much less frequent and all occurred after non-pulsatile experiments. These may be attributed to cell swelling or shrinkage or to the general loss of all types of cells.

The preliminary test counts provide such wide ranges of error that, even accepting the wide limits of error calculated above, conclusions based upon Quantimet cell counts must be drawn cautiously

and then verified by other methods. Both swelling and shrinkage of cells can be verified by measuring large numbers of cells under the light-microscope or by examining a smaller selection of cells with the electron-microscope. Further evidence is presented here as a result of electron-microscope observations.

ELECTRON-MICROSCOPY EXPERIMENTS

Introduction

In comparing the brains of dogs subjected to 1, 2 and 3 hours of non-pulsatile extracorporeal circulation, qualitative differences in light-microscope sections could only be suspected. There appeared to be no cellular damage after 1-hour perfusions or after 3 hours of anaesthesia. The objects of the electron-microscope studies were:-

(a) to verify the light-microscope results,

(b) to look for sub-cellular changes after 1-hour perfusions and after 3 hours of anaesthesia,

(c) to determine whether or not cell swelling or shrinkage could account for the changes in cell numbers recorded in the previous section,

(d) to attempt to demonstrate qualitatively progressive subcellular changes after 1, 2 and 3-hour non-pulsatile perfusions.

The brains of several mammalian species have been examined with the electron-microscope. Rat, cat and monkey brains have been most frequently used for descriptions of normal fine structure (e.g. Peters and Kaiserman-Abramof, 1969). Functional relationships between various brain regions have been studied in the cat by transection of axon fasciculi and sequential electron-microscopy of retrograde

synaptic degeneration (e.g. Guillery, 1965).

Dog brain is not suitable for either of these types of study and has received very little attention. The dendritic spine apparatus of dog sensori-motor cortex has been described by Gray and Guillery (1963). Van Breeman and Bowman (1966) have found the peculiar lamellar bodies in dog Purkinje cells. There has been no general description of the fine structure of dog brain or even of any region of the brain.

No previous report has described fine structural changes in the brain of any animal following extracorporeal circulation, but fine structural changes in the hearts of children undergoing open-heart surgery have been described by Keen and Dowlatshahi (1970) and Lee et al (1968) have described ultrastructural changes in three isolated dog brains.

Materials and Methods

Non-pulsatile extracorporeal circulation was established in beagle dogs as described previously. Table C47 lists the experiments performed and gives the pH and osmolarity of the primary fixative. Osmolarity was determined by a freezing point depression technique using a Fiske model H66 osmometer. After defibrillating the heart at the end of each by-pass, the brain was perfuse-fixed by the technique described in Appendix 2 except that the fixative was 6 l of 2.5% glutaraldehyde buffered to pH 7.3-7.5 with monobasic and

dibasic sodium phosphate buffer (Karlsson and Schultz, 1965).

When the perfusion-fixation was completed, the common carotid arteries and jugular veins were ligated and the head was removed. Approximately 1 hour later, $4mm^3$ samples of brain were selected from the frontal (left rectal gyrus), parietal (right cingular gyrus) and occipital (right posterior suprasylvian gyrus) cerebral cortex, the head of the right caudate nucleus, the left dorsal parietal nucleus of the thalamus and the cerebellar cortex to include molecular and granular layers of the vermis. The brain samples were placed in buffered glutaraldehyde and each sample was dissected to give 4 symmetrical $1mm^3$ blocks. The blocks of tissue from the cerebral cortex were trimmed so that 4 blocks included layers 1, 2 and 3, and a further 4 blocks included layers 4, 5 and 6. Thus the total number of tissue blocks obtained from each brain was 36, but usually only 10-15 of these blocks were sectioned.

The total time for primary fixation from the completion of perfusion-fixation to the first buffer rinse was 3.5 hours in each case. Subsequent processing was performed with the aid of an automatic shaking machine:-

1. Phosphate buffer overnight.
2. Phosphate buffer for 30 minutes.
3. 1% oxmium tetroxide in phosphate buffer for 2 hours.
4. Phosphate buffer for 1 hour.

5. Dehydrate through a graded series of ethanol 70%, 80%, 95% and 100% (3 changes) for 30 minutes each.
6. Three 30-minute changes of epoxy-propylene.
7. Epoxy-propylene and TAAB embedding resin (1:1) overnight.
8. Three changes of TAAB embedding resin for 8 hours, overnight and 8 hours respectively.
9. Embed in BEEM polythene capsules containing TAAB embedding resin, incubate at 37°C for 2 hours and polymerise at 60°C for 48 hours or more.

Sections 600-900Å thick were cut on a Reichert OmU2 ultratome or on an LKB 8800A ultratome III, double-stained with lead citrate (Reynolds, 1963) and uranyl acetate (Watson, 1958), and examined with a Philips EM 200 electron-microscope. Sections 1μ thick were cut on the same ultratomes for examination with the light-microscope. These sections were dehydrated by gentle heating, stained with 1% toluidine blue in 1% sodium tetraborate, dehydrated as before and mounted in Canada balsam. This is a modification of the method described by Trump et al (1961).

Results

The ultrastructure of normal dog brain.

Controls 126, 128 and 133.

Although the ultrastructure of dog brain has not been previously reported, for the sake of brevity and singleness of purpose, and in

order not to dissipate the reader's interest, a comprehensive description is not included here. Instead, anatomical structures relevant to the identification of cells and cell processes are described and selected normal structures are compared with similar structures undergoing pathological changes.

The surface area of an electron-microscope section is usually about $0.05mm^2$. Of this, no more than $0.01mm^2$ can be viewed at one time with the electron-microscope using standard 200-mesh grids. Despite this difficulty, laminae of the cerebral cortex and cell types can be distinguished with some certainty by their ultrastructural peculiarities. The $1mm^3$ tissue blocks were cut so that cortical layers 3 and 5, which contain the majority of pyramidal nerve cells, were included in separate blocks. Layers 2 and 4, which are mainly composed of cortical nerve cells, were also separated. Layer 2 also includes some pyramidal cells that can be differentiated from those of layer 3 by their relatively small size and by their close proximity to the almost acellular layer 1. At deeper levels of the cerebral cortex, the increasing density of medullated axons in layers 5 and 6 and the fusiform cells of layer 6 are useful features for orientation. Thick sections stained with toluidine blue provide additional information in doubtful cases.

The cerebral cortex. The criteria for distinguishing between pyramidal and cortical nerve cells in electron-micrographs of the mammalian brain have been established by Jones and Powell (1970). These criteria

have been incorporated in the following description of dog cerebral cortex.

As in light-microscope sections of the cerebral cortex, pyramidal cells may be recognised by their distinctive triangular outline and by the arrangement of their cell processes (Fig. C105). In electron-microscope sections cut perpendicular to the brain surface, each pyramidal cell displays a broad apical dendrite that is frequently visible for a considerable part of its length and one or two basal dendrites that are less frequently seen in continuity with the perikaryon. The axon hillock arises between the basal dendrites. The large, round nucleus has evenly dispersed chromatin and commonly has a few shallow indentations of its membranes. The cytoplasm contains numerous evenly distributed rosettes of free ribosomes. Rough surfaced endoplasmic reticulum is diffusely but not densely scattered throughout the cytoplasm. The cisternae become sufficiently aggregated to be recognisable as Nissl bodies only at the base of the apical dendrite. Golgi complexes, mitochondria, neurotubules, neurofilaments, non-specific granules and a few dense bodies and vesicles are present in the cell cytoplasm.

Except for the fusiform cells of layer 6, cortical nerve cells are usually round or oval in outline (Fig. C106). They frequently have only a single dendrite but sometimes several small dendritic processes arise from the cell surface. Both the dendritic processes and the axon hillock are more slender than those of pyramidal cells

and they make more acute connections with the perikaryon. The nucleus is usually deeply invaginated by cytoplasmic processes that are densely packed with free ribosomes. Cortical cells may sometimes be distinguished from pyramidal cells by their higher concentration of cytoplasmic organelles, particularly mitochondria, Golgi complexes and rough surfaced endoplasmic reticulum organised into conspicuous Nissl bodies. However, this is not a consistent difference in dog cerebral cortex.

The two cell types also differ in their dendritic ramifications and in the form of their synaptic relationships (Figs. C105, C106 and C107). Axodendritic contacts are the most frequent on both cell types, but whereas the majority of axon terminals contact the dendritic spines of pyramidal nerve cells they mostly contact the perikarya and dendritic shafts of cortical nerve cells. In both cell types the synapses on the perikarya and dendritic shafts are symmetrical. The axon terminals usually contain small, flattened or pleomorphic vesicles. Symmetrical axo-axonic synapses are found on the axon hillocks and initial segments of both cell types. Asymmetrical synapses are formed on the dendritic spines of pyramidal cells by axon terminals containing large spherical vesicles (Fig. C107). A spine apparatus is frequently associated with asymmetrical synapses. A peculiar feature in dog brain is that, in the occipital cortex, the cortical nerve cells have somatic spines that form symmetrical synapses with axon terminals. These are illustrated in the abnormal cell in Fig. C128.

The cerebral cortex has very little extracellular space when viewed with the electron-microscope. Spaces between adjacent cell processes are generally about 200Å wide. The dense arrangement of cerebral neuropil is shown in Fig. C107.

The cerebellar cortex

The structural organization of the cerebellar cortex is probably better understood than any other region of the mammalian brain. The cortex may be considered in two parts - granular and molecular layers.

1. The granular layer. In light-microscope sections stained by standard techniques, only the cell nuclei and medullated fibres are clearly visible in the granular layer, but electron-micrographs show that the volume of extracellular space is similar to that of the cerebral cortex and that the neuropil is equally dense.

The granular layer is mainly composed of a very large number of small granule cells and their dendritic processes (Fig. C108). Granule cells are round or oval in outline and have little cytoplasm (Figs. C109 and C110). The nucleus is oval and the nuclear chromatin is disposed in clumps mostly close to the nuclear membrane. Several short dendritic processes arise from each granule cell and a slender apical axon is subtended to the molecular layer where it bifurcates to form parallel fibres (Fig. C111). Nissl bodies are absent from the cytoplasmic rim but a few mitochondria, a small Golgi complex,

neurotobules, neurofilaments and occasional dense bodies may be found.

The regions of neuropil between the granule cell perikarya are called 'cerebellar islands' (Fig. C110). These are formed exclusively by cell processes. Granule cell dendrites and mossy fibre and Golgi cell axons form complex synaptic relationships in the cerebellar islands. These synaptic regions, referred to as 'rosettes', are distinguished by the presence of numerous dense, often elongated mitochondria in the axon terminals and by the large number of asymmetrical synaptic contacts that each terminal makes with granule cell dendritic processes.

The medullated axons of mossy fibres, climbing fibres, Purkinje cells and their recurrent collaterals are also seen as they pass through the granular layer (Fig. C109).

2. The molecular layer. In electron-microscope sections cut transversely across the folia, the most conspicuous feature to be seen is the very large number of circular profiles (Fig. C111). These are the parallel fibres of granule cell axons that have constricted sections containing axonic tubules and dilated sections making asymmetrical synaptic contacts with the spiny branchlets of Purkinje cell dendrites.

Purkinje cells are among the largest cells in the brain. The cell perikaryon is round or flask-shaped (Fig. C112). The nucleus

is large and usually round. Electron-micrographs often show deep invaginations of the nuclear membranes. The cytoplasm is densely filled with cytoplasmic organelles. Mitochondria are numerous, particularly close to the surface membrane. Granular endoplasmic reticulum, sometimes organized as lamellar bodies, and Golgi complexes are plentiful. Several dense bodies and multivesicular bodies can be seen in a dense matrix of free ribosomes.

Usually one, sometimes two and occasionally three primary dendrites are produced from the superficial cell surface (Fig. C111). The dendrites ramify in the plane perpendicular to the long axis of the folium to form secondary and tertiary smooth branches. The smooth branches are densely packed with canaliculi and longitudinally arranged cisternae of the granular endoplasmic reticulum.

Spiny branchlets arise from the smooth branches and make synaptic contacts with the parallel fibres (Fig. C111). Numerous spines are produced by each spiny branchlet. The branchlets contain numerous mitochondria and neurotubules.

The caudate nucleus

The head of the caudate nucleus is composed mainly of medullated and non-medullated axons (Fig. C113). The scattered nerve cells resemble cerebral cortical cells. Their nuclei are approximately round and they have deep invaginations of the nuclear membranes. The cell perikarya have only a thin rim of cytoplasm containing granular endoplasmic reticulum, Golgi complexes, mitochondria and

clusters of free ribosomes (Fig. C114). Some of the endoplasmic reticulum is arranged as agranular lamellae close to the cell surface. A few dense bodies and multivesicular bodies can be found in the cytoplasm.

Most of the synapses are axo-dendritic and asymmetrical (Fig. C113). The axon terminals contain round and ellipsoidal vesicles. A few axo-somatic synapses are present on each cell soma and these appear to be symmetrical with ellipsoidal vesicles.

Cells producing typical 9 + 2 cilia are present in the ependymal cell layer (Fig. C115).

The dorsal parietal nucleus

The structure of the dorsal parietal nucleus of the thalamus resembles that of the caudate nucleus but both cortical and pyramidal cell types are present (Figs. C116 and C117). In the dorsal parietal nucleus, the cortical and pyramidal types of cells are distinguished mainly by their nuclei and cell shapes, the cytoplasmic inclusions being similar in both types. As in the cerebral cortex, the nuclear chromatin is evenly dispersed and cortical type cells usually display deep invaginations of the nuclear membranes. The cytoplasm of both cell types is not dense, but it is particularly rich in small mitochondria, and both Golgi complexes and granular endoplasmic reticulum are plentiful. Dense bodies are common.

Many synapses are present in the neuropil and all of these appear to be asymmetrical with spherical synaptic vesicles. Much

of the region is occupied by heavily medullated small calibre axons containing few cytoplasmic organelles.

The white matter

The white matter of cerebral and cerebellar cortices and of the caudate and dorsal parietal nuclei is composed mainly of medullated axons and neuroglial processes (Fig. C118). At suitable magnifications of thin sections, the periodic structure of myelin is readily visible. Interspersed between the medullated axons are the cell processes of astrocytes, oligodendrocytes and occasional microglia.

Neuroglial cells

The three neuroglial cell types - astrocytes, oligodendrocytes and microglia - can be distinguished in electron-micrographs, but their appearances are very much dependent upon the quality of fixation and upon the fixatives employed.

In this study, astrocytes were easily identified by their small, round to oval nuclei with evenly dispersed chromatin (Figs. C105 and C118). The cytoplasm is poor in organelles. It contains several small mitochondria, a few dispersed lamellae of granular endoplasmic reticulum, a small Golgi complex, multivesicular bodies, non-specific granules and glial filaments. The filaments are sometimes considered to be characteristic of astrocytes. They are usually arranged in dense bundles near the emergence of cell processes from the perikaryon. Both protoplasmic and fibrous astrocytes contain

glial filaments, but there are many more in the processes of fibrous astrocytes. Both astrocyte forms have glycogen granules in their terminal processes (Fig. C107). Protoplasmic and fibrous astrocytes may be distinguished by the more frequent branching of the processes of protoplasmic astrocytes and by their locations. Protoplasmic astrocytes are most frequently seen in the grey matter where many of them are neuronal satellites. Fibrous astrocytes are found mainly in the white matter.

Oligodendrocytes are characterised by a high electron opacity of the cytoplasm which is due to the high numbers of cytoplasmic organelles and to a dense granularity of the matrix (Figs. C105, C109 and C117). The cytoplasmic organelles include large numbers of small mitochondria, granular endoplasmic reticulum, a limited number of short Golgi cisternae, multivesicular bodies and usually several dense bodies. Oligodendrocytes never possess glial filaments in their normal state, but tubules may be present close to the nucleus. The latter is small, usually eccentrically placed in the perikaryon and the chromatin is aggregated into clumps.

Microglial cytoplasm has a similar electron density to that of oligodendrocytes but the predominant organelles are different (Fig. C122). Microglial cytoplasm has a globular and vesicular appearance and it may contain large numbers of dense bodies. Golgi complexes are frequently seen, but neither filaments, tubules nor glycogen granules are present in the cytoplasm. The nucleus has a

similar form to that of the oligodendrocyte but tends to be narrower. An indefinite nuclear membrane and large numbers of wide nuclear pores are distinctive features. The cell surface membrane is not smooth like that of the oligodendrocyte, but has indentations and a high electron density. The immediate pericellular region is sometimes devoid of neuropil.

Synopsis of cell changes detected by electron-microscopy

Cell changes of two distinct types were found in these experiments. In one type a loss of cytoplasmic organelles was associated with cell swelling and, in the other type, an increase in the number of cytoplasmic organelles was associated with cell shrinkage.

Subcellular changes associated with cell swelling were seen in the brains of dogs subjected to 3 hours of anaesthesia with the thorax open. Pathological changes included nerve cell and astrocyte cytoplasmic vacuolation, some mitochondrial swelling, expanded Golgi cisternae, swollen astrocyte processes and the formation of peripheral clear spaces in the cytoplasm of nerve cells (Figs. C119, C120 and C121).

Evidence of cell and organelle swelling was found in the brains of all of the dogs subjected to non-pulsatile perfusion. In some respects these changes were no worse after 3-hour than after 1-hour perfusions. On the contrary, mitochondria in the perikarya of nerve cells in the cerebral cortex were more swollen after 1-hour than

after 3-hour perfusions. However, peripheral clear spaces were not evident in the perikarya of nerve cells after 1-hour perfusions, but they were found after 2-hour perfusions and they were more numerous after 3-hour perfusions. A few peripheral clear spaces were seen in the nerve cell perikarya of the 3-hour anaesthetic group of dogs. Severe loss of astrocyte cytoplasm was noted with swollen astrocyte processes after all perfusions (Figs. C120 and C122).

Cells with increased numbers of cytoplasmic organelles were seen after 3 hours of anaesthesia and after all of the perfusions. After 1-hour perfusions, there was a mild increase in the number of dense bodies in the perikaryal cytoplasm and in the proximal dendrites (Fig. C123). Increased numbers of dense bodies and increased amounts of Golgi cisternae and granular endoplasmic reticulum were found in a few nerve cells in the cerebral cortex and in one Purkinje cell after 2-hour perfusions (Fig. C124). The nuclear membranes of some of these cells had become irregular in outline. Nuclear pores were prominent and stacks of granular endoplasmic reticulum were present in and close to the nuclear invaginations.

Dark cells were seen in the cerebral cortex and dorsal parietal nucleus of one dog subjected to 3 hours of anaesthesia with the thorax open (Figs. C125 and C126). The cytoplasmic density of these cells was increased by considerable proliferations of the granular endoplasmic reticulum, free ribosomal rosettes and non-specific granules. Golgi

complexes and dense bodies were also increased in numbers. The nuclear membranes were crenellated. One satellite cell had become so dark that its organelles could not be distinguished.

Even greater proliferations of cytoplasmic organelles were seen in the occipital and parietal cortex of one dog subjected to 3 hours of non-pulsatile perfusion (Figs. C127 and C128). In addition to the changes described in the previous paragraph, the cell surface membranes had become scalloped and the cells appeared to be shrunken. Axonal and dendritic profiles in the nearby neuropil were shrunken and the extracellular spaces were widened. The latter changes were most conspicuous close to blood vessels (Fig. C129).

Aggregates of dense bodies could be seen in oligodendrocytes and pericytal microglia, particularly after 3-hour perfusions (Fig. C130), and phagocytic cells could be seen in the Virchow-Robin spaces after all experimental procedures (Fig. C131).

Experiment reports

3-hours anaesthesia experiments 146, 148 and 153.

During continuous nitrous oxide-halothane anaesthesia with the thorax open, each dog's AP declined steadily. The decreases in AP were 78 to 52mm Hg in dog 146, 75 to 42mm Hg in dog 148 and 85 to 60mm Hg in dog 153. Increasing the concentration of inhaled halothane from 0.5% to 1.0% caused a rapid fall of 2-5mm Hg and the AP did not increase when the halothane concentration was returned to 0.5%.

During perfusion-fixation of dog 153, the aortic cannula withdrew into the left ventricle. Microscopic inspection of the exposed brain surface showed that several very small blood vessels in the cerebral cortex still contained blood. However, the entire brain appeared to be excellently fixed as did all of the brains in the series.of experiments for electron-microscopy.

All three of the brains of dogs subjected to 3 hours of anaesthesia haddeveloped extensive subcellular damage. The cytoplasm of both pyramidal and cortical nerve cells was frequently seen to be severely vacuolated (Fig. C119). This was most prominent in layers 2 and 3 of the frontal and occipital regions of dogs 146 and 153, but it was restricted to layer 6 of the parietal cortex in dog 148. The vacuoles were located in the perikarya, particularly close to the surface membrane, and in the proximal dendritic shafts. In places, especially in the superficial layers of the frontal cortex, peripheral clear spaces had developed in nerve cell perikarya (Fig. C121). Astrocyte vascular end-feet appeared to be swollen and to have lost their contents in some places.

The cerebellar neurons were less severely affected, but the cytoplasm of granule cells contained swollen and vacuolated mitochondria which were noticed, only occasionally in the cerebral cortex (Fig. C120). Purkinje cell perikarya and mossy fibre rosettes also had a few vacuolated mitochondria.

The caudate and dorsal parietal nuclei were only slightly affected by mitochondrial swelling, but there was much intra- and extra-cellular vacuolation in the dorsal parietal nucleus. Some cells in this region had expanded cisternae of the Golgi complexes.

The cytoplasm of some pyramidal and cortical nerve cells in the occipital cerebral cortex and dorsal parietal nucleus of dog 148 had become 'dark' due to substantial increases in the amounts of granular endoplasmic reticulum, free ribosomes and non-specific granules (Fig. C125). These cells had become distorted in shape and the nuclear membranes were crenellated.. Synaptic processes close to these cells were increased in electron opacity, and astrocyte processes were swollen. The nuclear membranes of satellite oligodendrocytes had become deeply invaginated. Fig. C126 shows a nerve cell with increased cytoplasmic density. Its satellite cell has become so electron opaque that it is impossible to distinguish cytoplasmic organelles.

1-hour non-pulsatile experiments 129 and 151.

These two perfusions were uneventful, but both dogs became hypotensive at the time of defibrillation (lowest APs 8mm Hg in dog 129 and 19mm Hg in dog 151). After defibrillation, homologous blood transfusion elevated the AP to 30mm Hg in both cases, but in dog 129 the arterial blood remained desaturated up to the time of perfusion-fixation.

Both brains appeared to be well fixed.

Pathological changes in the cerebral cortex were characterised by swollen mitochondria. No cell layer was spared and both nerve cells and astrocytes were affected. Swollen mitochondria were usually round or oval in shape and the cristae were either disrupted or retracted, so that in severe cases only a spherical double-membraned sac remained (Fig. C120). The normally electron-opaque mitrochondrial matrix had been lost.

Granular endoplasmic reticulum appeared to be normal in cerebral nerve cell perikarya but Golgi cisternae were mildly expanded into membrane-bound vesicles (Fig. C120). Dense bodies were numerous in the cytoplasm.

The mitochondria present in pre-synaptic terminals were almost always normal, but the synaptic vesicles were sometimes clumped together near the centre of the terminal or close to the synaptic cleft (Fig. C132).

Astrocyte processes in the cerebral cortex were mildly expanded and deficient in glycogen granules. Several interstitial astrocytes were almost devoid of cytoplasmic organelles (Fig. C122). The chromatin of all cell nuclei in the cerebral cortex of dog 129 was aggregated into clumps and this made the distinction between neuroglial cell types more difficult, but the shapes of the nuclei, the number and form of cell processes and the few remaining organelles indicated that the predominantly affected neuroglial cells were protoplasmic

astrocytes. Fig. C122 shows two cells that have been tentatively identified as a normal microglial cell and a degenerating astrocyte satellite to a pyramidal nerve cell. The astrocyte is swollen. It contains a nucleus with aggregated chromatin and a tangentially sectioned nuclear membrane in which nuclear pores are visible. The cytoplasm is much thinner than normal and all of its organelles are degenerating, with the possible exception of the few isolated lamellae of granular endoplasmic reticulum. The cytoplasm of the microglial cell looks relatively healthy. Cytoplasmic globules and vesicles are conspicuous. Short tubules radiate from the surface membrane of the microglial cell into the cytoplasm of the astrocyte.

Nuclear chromatin was aggregated in the Purkinje and Bergmann cells of the cerebellum of dog 129. Aggregated chromatin is normal in granule cell nuclei. The cerebellar neuropil of both brains was generally better preserved than cerebral neuropil, but Purkinje cell dendrites, climbing fibre terminals, granule cell terminals and astrocyte processes had reduced cytoplasmic contents (Fig. C132). Granule cell perikarya and mossy fibre rosettes appeared to be normal.

The neuropil of the caudate and dorsal parietal nuclei was not severely damaged but mitochondrial vacuolation was extensive in the nerve cell perikarya. Large vesicles, identified as degenerating mitochondria by their double membranes, were seen in the neuronal perikarya of the dorsal parietal nucleus.

Vascular cells were well preserved in all regions of the brain,

but astrocyte and oligodendrocyte vascular end-feet were mildly expanded and devoid of cytoplasmic organelles. Large granular and vesicular dense bodies were seen in cells located in the Virchow-Robin spaces (Fig. C131).

2-hour non-pulsatile experiments 134 and 135

Experiment 134 was uneventful. The heart fibrillated spontaneously when the perfusion started, but it was easily defibrillated at the end of the perfusion. The heart of dog 135 was distended throughout the period of extracorporeal circulation due to regurgitation past the pulmonary artery cannula into the right ventricle. Large volumes of bronchial fluid were exuded from the trachea during the last 30 minutes of perfusion. After perfusion-fixation, both brains appeared to be well fixed. The lungs of dog 135 were oedematous and congested.

These two brains exhibited rather less severe mitochondrial alterations than the two brains obtained after 1-hour perfusions. Much of the cerebral cortex appeared to be entirely normal (Fig. C133). Severely damaged neuropil like that described in dogs 129 and 151 was seen only in the deep cortical layers (4, 5 and 6) of dog 134. In these regions, large cytoplasmic vacuoles were seen in some nerve cells (Fig. C119). Other nerve cells displayed slight peripheral clear spaces in the cytoplasm.

Dense bodies appeared to be more numerous than normal in the cerebral nerve cell perikarya of both brains. In dog 135, one cortical nerve cell in the occipital cortex and a Purkinje cell

appeared to have slightly increased cytoplasmic densities due mainly to increased numbers of dense bodies and some increases in Golgi cisternae and granular endoplasmic reticulum (Fig. C124). The nuclear membranes had become mildly crenellated and, in the Purkinje cell, stacks of granular endoplasmic reticulum were located in and close to the nuclear invaginations.

Vacuolar degeneration of astrocyte processes was minimal in the cerebral neuropil, but a few astrocyte processes were mildly swollen. Perivascular spaces were restricted to large arteries and veins. Free phagocytic cells were not found in the Virchow-Robin spaces, but granular and globular dense bodies were not uncommon in pericytal cytoplasm and binucleated astrocytes were seen near one blood vessel in the parietal cortex (Fig. C133).

Degenerative changes were mild in the cerebellar neuropil. Swollen mitochondria could be seen in the mossy fibre rosettes and in the smooth branches of Purkinje cell dendrites. Some granule cells had suffered a slight peripheral loss of cytoplasm. Increased numbers of dense bodies were observed in oligodendrocytes of the granular layer. There appeared to be an increase in the number of Bergmann cells, particularly near blood vessels, though it is noted that tissue prepared for electron-microscopy is not satisfactory for estimating cell population changes (Fig. C134). Dense bodies were numerous in the cerebellar pericytes.

The neuropil of the caudate and dorsal parietal nuclei appeared

to be well preserved. However, many nerve cell perikarya in both nuclei of both brains displayed peripheral clear spaces in the cytoplasm. Mitochondrial vacuolation was seen more frequently in and close to the nerve cell perikarya than in the distal processes. Some astrocyte processes were swollen to the point of disruption of the surface membrane. Dense bodies were present in the cytoplasm of oligodendrocytes and pericytes though they were not numerous.

3-hour non-pulsatile experiments 136 and 137

DeBakey pump arterial pulse amplitudes of up to 20mm Hg were recorded in experiment 136. The significance of these pulses was discussed in the section on physiological data. Because of diffuse bleeding into the thorax and poor venous returns, it was necessary to add 1500 ml of homologous blood to the reservoirs during perfusion. However, the AP remained between 60mm Hg and 120mm Hg. The perfusion of dog 137 was also successful. The heart frequently reverted to sinus rhythm spontaneously and required repeated fibrillation shocks for the elimination of ventricle contractions. An oxygen cylinder expired during the perfusion, but the dog breathed air for the 3-4 minutes taken to change the cylinder and there were no apparent detrimental effects.

Both brains appeared to be well fixed.

Wide peripheral clear spaces had developed in some cerebral nerve cell perikarya and in some astrocyte cell bodies (Fig. C121).

Mitochondrial vacuolation was mild in dog 136 and much more widespread in dog 137. Both vacuolated mitochondria and dense bodies could be found in dendritic spines.

In dog 137, the cytoplasm of some cerebral nerve cells in the parietal and occipital regions was seen to be increased in density. Fig. C127 shows two 'dark' cortical nerve cells in layer 2 of the occipital cortex. The more central nerve cell is shown at a higher magnification in Fig. C128. The increase in cytoplasmic density is due to the proliferation of granular endoplasmic reticulum, ribosomal rosettes and non-specific granules. The mitochondria are small and dense and have assumed tortuous shapes. The nuclear membrane is more deeply invaginated than normal and free ribosomes are abundant in and close to these invaginations. Dense bodies and Golgi complexes also appear to be more numerous than normal. Synaptic regions appear to be normal except for the higher electron opacity of synaptic terminals. Axonal and dendritic profiles in the surrounding neuropil appear to be shrunken and the extracellular spaces are widened. The scalloped appearance of the nerve cell membrane suggests that the nerve cell may also be shrunken.

Shrinkage of nerve cell processes and widened extracellular spaces were most conspicuous and most frequently encountered close to capillaries (Fig. C129).

Swollen astrocyte processes were seen in the cerebral cortex

of both brains though they were not common. Dense bodies appeared to be generally increased in numbers especially in oligodendrocytes and microglia. Pericytal microglia were seen in Virchow-Robin spaces and they were not enclosed in the basement membranes.

In the cerebellum, cell perikarya of all types showed only mild mitochondrial vacuolation and the neuropil of the molecular layer was well preserved except for Purkinje cell dendrites (Fig. C135). Primary smooth branches and spiny branchlets were only slightly affected but there was a marked reduction of cytoplasmic organelles in the secondary and tertiary smooth branches. In these, mitochondria were fragmented, neurotubules and neurofilaments were disarranged and extensive clear spaces were seen to contain only membrane fragments and large membrane-bound vacuoles. Bergmann fibre processes in apposition to the Purkinje cell dendrites were swollen close to the dendritic clear spaces. The extracellular space was enlarged in the granular layer between climbing and mossy fibres and near blood vessels.

Artefacts

Membrane configurations bearing no resemblance to cytoplasmic organelles were seen in all of the brains. These are well known in electron-microscopy as 'myelin figures' because their electron-opacity is similar to that of myelin (Figs. C118 and C136). Myelin figures were seen in a few nerve cell processes and in astrocyte

processes in most sections. They were particularly conspicuous and extremely dense in the astrocyte processes in the white matter of cerebral and cerebellar cortices (Fig. C118). In these locations, the myelin appeared to produce globular, bulbous and whorled extensions from the surface into the fibrous astrocyte processes. A recent publication by Schultz and Case (1970) claims that myelin figures can be avoided by fixing the brain with 19% glutaraldehyde as a preliminary to fixation with the usual 2-5% concentration.

The 'washed out' appearance and vacuolation of astrocyte processes have been described in this section as pathological changes. However, it must be admitted that these changes were also occasionally seen in control brains (Fig. C106). This warning also applies to mitochondrial swelling.

Correlation of results obtained by light- and electron-microscopy

Additional information about the pathological nerve cell changes was sought by light-microscopical examinations of toluidine blue-stained sections cut from tissue blocks embedded in epoxy resin for electron-microscopy. The standard techniques for staining these sections are unsatisfactory because of the poor differentiation of neural tissues embedded in epoxy resin and because the stain fades after mounting.

Fig. C137 shows normal cells in layer 4 of the frontal cerebral cortex. Endothelial and neuroglial nuclei and the nucleoli of nerve cells stain deeply with toluidine blue. The deep invaginations of

cortical nerve cell nuclear membranes are clearly revealed. Myelin is also well stained but other structures are poorly differentiated.

The formation of microvacuoles in the neuropil and in the peripheral cytoplasm of nerve cells following 3 hours of anaesthesia is illustrated in Fig. C138. Rarefied neuropil was found in the molecular layer of the cerebellum, and some Purkinje cells had developed slight peripheral clear spaces (Fig. C139).

Darkly-stained cells (Fig. C140) were seen in the occipital cortex of dog 148 (3 hours anaesthesia) and one Purkinje cell was also darkly-stained. Darkly-stained nerve cells were also seen in the parietal and occipital cortex of dog 137 after a 3-hour non-pulsatile perfusion. The neuropil was seen to be vacuolated especially close to dark nerve cells and capillaries. Fig. C140 shows the dark nerve cell and nearby neuropil illustrated in the electron-micrograph Fig. C128.

Slight pericapillary spaces were seen after 1-hour and 2-hour perfusions and these were wide after 3-hour perfusions (Figs. C141 and C142).

Discussion

It appears that the results obtained by light- and electron-microscopy are compatible. Intracellular swelling of cerebral nerve cells was not detected by conventional light-microscopy except in one case (dog 108). However, it was seen in the cerebellar Purkinje cells and it was suspected in the cerebral cortex as a result of

Quantimet cell counts. Nerve cell cytoplasmic vacuolation and peripheral clear spaces seen in electron-micrographs following 3 hours of anaesthesia and following non-pulsatile perfusions could be due to cell swelling or to the loss of peripheral cytoplasmic organelles. The latter explanation is to be preferred on morphological grounds, for the nerve cells with peripheral clear spaces did not appear to be significantly swollen. However, no membrane fragments were seen in these spaces and there is no reason for expecting peripheral organelles to be lost before more central ones. On the other hand, the formation of peripheral clear spaces can easily be explained as being the result of cellular water uptake following ischaemia (Van Harreveld, 1957).

The nerve cells that stain darkly with toluidine blue in epoxy resin brain sections can be identified as cells seen to have greatly increased numbers of cytoplasmic organelles in electron-microscope sections. These cells may be related to those undergoing ICC in paraffin wax sections and it seems likely that the increased numbers of cytoplasmic organelles and granules are responsible for the diffuse PAS-positive reactions.

Finally rarefied neuropil was identified by both light- and electron-microscopy and it was easily seen in toluidine blue-stained epoxy resin sections. Its occurrence in light-microscope sections appears to be due to both the swelling of astrocyte processes and

the formation of wide extracellular spaces by the shrinkage of cell processes.

Presuming that these relationships are correct, it appears that cells and cell processes may either swell or shrink as a result of non-pulsatile extracorporeal circulation. Mitochondrial swelling and cytoplasmic vacuolation were the earliest signs of swelling. Astrocyte processes were definitely swollen but the electron-microscope evidence for neuronal swelling was less conclusive.

Nerve cell shrinkage appears to be associated with increased density of the cytoplasm. A proliferation of dense bodies was the earliest sign of this change. Increased numbers of Golgi complexes and granular endoplasmic reticulum followed 2-hour and 3-hour perfusions and were associated with increased numbers of ribosomal rosettes and non-specific granules. The end product was a distorted, densely-packed cytoplasm and a crenellated nucleus. The extracellular spaces seen around ischaemic nerve cells in light-microscope sections appear to be due to the shrinkage of the cell and nearby processes and not to swollen astrocyte processes.

The possibility that cell changes involving swelling and shrinkage are not exclusive is suggested by the electron-micrograph Fig. C121. This micrograph shows a nerve cell that has developed a wide peripheral clear space in the presence of a slight increase in endoplasmic

reticulum. Both swelling and shrinkage changes can be found in the same brain. It was a surprise to find them both in the brain of one dog subjected to 3 hours of anaesthesia but with no extracorporeal circulation. It appears that the effects of anaesthetics upon the cerebral microcirculation and upon neuronal fine structure deserves some attention. It may be that swelling and shrinkage reactions can be caused by the reduced cardiac output and hypotension resulting from prolonged nitrous oxide-halothane ventilation (Raventós, 1956). It is not possible that these factors could explain similar changes seen in the brains of dogs subjected to non-pulsatile extracorporeal circulation because anaesthetic administration was only intermittent, the differences between pulsatile and non-pulsatile flow rates were not significant and because the non-pulsatile animals did not become hypotensive (see physiological data).

These findings indicate that cellular damage is progressive and that the nerve cell changes are graded in severity depending upon the duration of extracorporeal circulation. Thus the hypothesis that the nerve cell changes detected by light-microscopy genuinely represent pathological cell changes acquires support. Of course, the possibility that artefactual changes are present in both light- and electron-microscope results is not denied. This is more likely to apply to cell changes involving swelling than those in which shrinkage followed the proliferation of cytoplasmic organelles,

since the evolution of swelling was not clearly demonstrated.

Enthusiasm for these critical conclusions is dampened by the caution that the requirements of electron-microscope technique introduce a large amount of selectivity. Therefore, much more than in light-microscopy, conclusions drawn from results obtained with the electron-microscope on relatively small tissue samples are not necessarily applicable to the whole brain.

Nevertheless, the electron-microscopy results complement those obtained by light-microscopy so that the suggested pattern of evolution of changes shown in Fig. C90 can be modified to the form shown in Fig. C143. Artefacts are more likely to complicate the left hand side (cell swelling) than the right hand side (cell shrinkage) of the diagram.

COMPARISON OF PERFUSION-FIXATION DATA

Introduction

The aim of this section is to explore the possibility that the variety of histological and electron-microscopical results previously described may be attributed to differences in the quality of fixation.

Materials and Methods

The periods during which the acacia-saline wash and fixative were flowing were timed in each experiment and records of AP were obtained as described in the section on physiological data and in Appendix 2. The APs were recorded during perfusion-fixation for most experiments after experiment number 94, but technical difficulties prevented AP recording during some recovery experiments.

At the time when the tissue blocks were being prepared from the perfuse-fixed brain, the quality of fixation was assessed according to the following schemes:-

Scheme for light-microscopy

Grade	Quality
1	Excellent - uniformly hard and white.
2	Very good - occasional blood vessel contains blood but tissue hard and white.
3	Good - slight pinkness in some regions.

Grade	Quality
4	Patchy - regions of soft, pink tissue.
5	Poor - generally soft and pink.

Scheme for electron-microscopy

Grade	Quality
1	Excellent - uniformly hard and pale yellow.
2	Very good - occasional blood vessel contains blood but tissue uniformly hard and pale yellow.
3	Poor - generally soft and pink.

Two brains perfuse-fixed for light-microscopy were classified as grade 5 and four brains fixed for electron-microscopy were classified as grade 3. The poor results obtained in all of these six brains were due to technical errors in the perfusion-fixation procedure. These brains have not been included in the following results.

Results

The results are listed in Tables C48 to C50 and fluctuations in AP are shown in Figs. C144 to C153. In the tables, the values of AP listed under 'cannulation' were those recorded 10 seconds before the acacia-saline wash commenced i.e. after the aortic cannula had been inserted. The values listed under 'wash' and 'fix'

are arithmetic means calculated from measurements made at 10-second intervals during the fixation procedure. Total ICC was obtained by vertical addition of the values listed in Tables C25 and C26.

The mean values for flow rates of the two solutions and APs recorded before and during perfusion-fixation were statistically analysed by Student's t-test to determine whether or not there were any significant differences between the control and experimental groups in the non-recovery series of experiments. The probabilities that the observed differences were due to chance are shown in Table C51. The only values that approached the 5% significance level were those calculated from the measured flow rates of fixative during 3-hour pulsatile light-microscopy experiments.

A cursory comparison of fixation grades and total ICC in Tables C48 and C49 reveals that there is a relationship between these two factors. The use of arbitrary scales prohibits correlation and regression analysis, but it is manifest that the highest values of total ICC were allocated to brains that were less than excellently fixed. The quality of fixation does not appear to have been determined by the flow rates of wash or fixative. It is possible that the mean AP during cannulation had some influence on fixation because all of the animals with relatively high APs at this time were well perfuse-fixed. On the other hand, low initial APs did not prevent excellent fixation in experiments 100, 111, 112, 144, 149 and 156.

No physiological factor has been implicated in perfusion-fixation for electron-microscopy since, with one exception, all of the brains were judged to be excellently fixed and there were no significant differences between control and experimental flow rates or APs.

Figs. C144 to C153 show fairly common patterns of AP changes during perfusion-fixation. The AP increased rapidly when the flow of acacia-saline commenced but declined steeply when the venous clamp was removed. It eventually settled to a value between 20 and 60mm Hg, sometimes increasing slightly when the acacia-saline was replaced by fixative (indicated by x on each record). This common pattern of AP changes was not obviously affected by the type of flow employed during the previous perfusion for extracorporeal circulation.

Discussion

The results presented above show that there was no consistent relationship between the quality of fixation and the incidence of ICC in the brains of dogs previously subjected to extracorporeal circulation. Furthermore, the quality of fixation was not dependent upon the flow rates of wash or fixative within the ranges of values employed, nor were the APs recorded during cannulation and perfusion-fixation related to fixation quality.

The experimental brains did not differ significantly in respect to any physiological factor except that, in the light-

microscopy series, the flow rates of fixative were lower following 3-hour pulsatile perfusions than in the controls. However, it was noted that the brains tended to be less well fixed following non-pulsatile than following pulsatile perfusions for light-microscopy. This factor may have augmented a difference in the incidences of nerve cell changes, though it was not an exclusive cause of them.

That the brains fixed with glutaraldehyde for electron-microscopy were more consistently well fixed than those fixed with Susa for light-microscopy may have been due to the use of larger volumes of fixative and/or to the rapid penetration of glutaraldehyde into brain tissue (Schultz and Case, 1970).

Statistical fallacy number 3 relating to statistical analyses of low numbers of experiments is relevant to these conclusions (Physiological data, p. 61).

Introduction

Mural thrombi were found in light-microscope sections of the brains of dogs 98 and 144. Both of these dogs had survived for 1 week following non-pulsatile perfusions. Focal lesions are usually associated with circulatory emboli but the aetiology of focal lesions was not apparent in brain sections. Further investigations were undertaken in an attempt to identify the causative factors. These investigations are still in progress but some convincing results have been obtained from the preliminary exploratory experiments described here. The potential factors responsible for cerebral emboli are fat globules, aggregates of blood cells and gas bubbles.

1. Fat globules

Materials and methods

Frozen sections 10-12μ thick were obtained from the cerebral cortex to include the parasagittal regions, and from the mid-thalamus and internal capsule. Sections were obtained from all of the animals in the light-microscopy series and stained with a saturated aqueous solution of Sudan III plus IV.

Results

The normal appearance of cerebral cortex is shown in Fig. C154. Glial processes, particularly vascular end-feet were Sudanophilic in

all brains. Large Sudanophilic globules were found among the medullated fibres in each of the animals that had survived for 1 week (Fig. C74). However, similar globules were seen in one of the control brains.

2. Aggregates of blood cells

Materials and methods for in vitro experiments

Four in vitro experiments were performed to evaluate the effect of continuous recirculation of blood upon cellular aggregation. The circuit shown in Fig. C155 was used for these experiments. The blood was circulated by a DeBakey roller pump set just occlusively. The tubing was 6mm internal diameter PVC and the pump cuff was also 6mm internal diameter. The capacity of the PVC reservoir was 240 ml. The inlet tube to the reservoir was extended with PVC tubing to 7cm from the base of the reservoir, i.e. so that it was approximately half-way down the column of blood. A 6mm sampling tap was positioned with the outlet vertical. The transducer of a Doptone ultrasonic flow detector (Smith Kline Instrument Company) was placed on the tubing close to the DeBakey pump outlet so that the angle subtended by the transducer to the tubing was 90°. A good contact between the transducer and the tubing was ensured by rigidly securing the transducer with tape and by the application of an ultrasonic conducting gel to the interface. The ultrasonic flow detector was calibrated in the amplitude mode to produce a 2cm upward deflection

on a Mingograaf 81 pen recorder for a 100mV input, and the sensitivity of the AC-DC converter was adjusted so that 1-2 random signals/second were recorded during zero flow through the tubing.

Large mongrel dogs were used as blood donors. One donor dog was used for each experiment. Total exsanguination under sodium thiopentone anaesthesia was performed by cannulating one femoral artery. Each dog yielded 800-1000ml of blood which was preserved in 500ml capacity Fenwall blood packs (Baxter Laboratories Limited) at 4°C until required. Each blood pack contained 75ml of anticoagulant constituted as 0.8% citric acid, 2.2% sodium citrate and 2.45% dextrose monohydrate in water (ACD). Heparin, 0.5mg/100ml blood was added before use.

The circuit was flushed with 0.9% sodium chloride before priming with 800ml of heparinised ACD blood. The time intervals between collecting the blood from the donor dogs to priming the circuit were 48 hours in experiment 1, 68 hours in experiment 2 and 3 hours in experiments 3 and 4.

The blood was circulated for 15 minutes before taking the first sample and records. Thereafter, blood samples were taken every 10 minutes, and doppler records and temperature measurements were made every 5 minutes.

Blood samples (1.5ml) were taken from the sampling tap into plastic syringes and passed into plastic tubes each containing 1mg

of dipotassium ethylenediamine tetra-acetic acid (EDTA). Whole blood samples taken from the blood packs and from the mixed prime were examined for red cell aggregates. A 0.02ml alliquot of blood was taken from each sample and placed in 0.8ml of 3% cocaine in 0.2% sodium chloride to lyse the red cells for platelet counting. Platelets were counted in a Neubauer counting chamber using transmitted light and an overall microscope magnification of 400-times.

Doppler records were obtained immediately after blood sampling on each occasion and at other times as required during experiments 3 and 4. The AC-DC converter was not available for experiments 1 and 2 and results were obtained by counting the characteristic echoe 'chirps' in the audio signal.

Blood temperature was regulated by altering the water temperature in the heat exchanger. Blood temperature was measured by a mercury bulb thermometer placed in the reservoir so that the bulb was approximately at the middle of the column of blood.

A pyrimidopyrimidine compound, 2,6-bis(diethanolamino)-4-piperidino-pyrimido[5,4-d] pyrimidine (RA 233, Boehringer Ingelheim Limited) was added to the blood at various stages of the procedure to determine its effect on platelet aggregation. A solution of RA 233 was made by dissolving 100mg of RA 233 in 2.5ml 0.1N hydrochloric acid and the volume was made up to 10ml with distilled water. After filtering, it was added to the blood to give a concentration

of 6mg RA 233/100ml blood. The pyrimidopyrimidine was added to the blood after 25 minutes of circulation in experiment 1 and after 105 minutes of circulation in experiment 4. In experiments 2 and 3, pyrimidopyrimidine was added to the blood before priming the circuit and a further dose was administered after 25 minutes of circulation in experiment 3.

Results

Red cell rouleaux were identified in all samples of blood taken from the blood packs before priming. However, none were seen in blood samples taken from the mixed prime i.e. after 15 minutes of circulation. Platelet aggregates were also present in samples taken from the blood packs in experiments 1 and 2, but not in experiments 3 and 4. Both red cell rouleaux and platelet aggregates were removed by passing the blood sample through a 10ml plastic filter holder packed with 2g of nylon wool. However, the platelet count was reduced from 312,000 to 204,000/ml by this method. Fig. C156 shows blood collected from the nylon filter.

Fig. C157 shows aggregates of platelets formed during *in vitro* blood circulation in experiment 1. In Fig. C158 the percentage of platelets involved in aggregates of 6 or more platelets is plotted against the duration of blood circulation for all four experiments. Figs. C159 to C162 relate the numbers of aggregates consisting of 6 or more platelets to the blood temperature, doppler frequency and

duration of blood circulation in individual experiments. Both audio and electrical signals were recorded during experiment 3. Subsequent analysis of the records showed that the best correspondence with the audio signals was given by the pen recorded signals 6mm or more in amplitude. These will be called 'high amplitude' doppler signals.

Most of the aggregates included in the figures consisted of 6-15 platelets in irregular or approximately spherical groups. In spherical aggregates of more than 10 platelets, counting was more difficult than in smaller aggregates, but reasonable accuracy could be obtained by adjusting the objective focus. Focus adjustment is normally not permitted in platelet counting because the calculations of sample volume are dependent upon the focal depth of the objective, so itwwas necessary to reset the focus after counting platelets in large aggregates. Many small groups of 2-5 platelets were seen in all blood samples. In pairs and triplets, it was impossible to determine whether the platelets were aggregated or simply in apposition to each other. Since the maximum diameter of these small groups of platelets did not exceed 7μ, it was concluded that they could have only a minor responsibility for microvascular occlusion during extracorporeal circulation. Therefore, only aggregates consisting of 6 or more platelets were considered to be important, though the small aggregates were counted. The numbers of aggregates composed of 2-5 platelets were as shown below. The proportions of platelets

involved in these small aggregates are shown in parentheses:-

	No. of aggregates/ml blood
Experiment 1 :	34-108 aggregates (43.0-45.9%)
Experiment 2 :	18-44 aggregates (22.7-24.5%)
Experiment 3 :	14-54 aggregates (14.3-37.0%)
Experiment 4 :	14-46 aggregates (8.5-55.2%)

The total numbers of circulating platelets decreased in all four experiments. The decreases were from 530,000 to 422,000/ml after 190 minutes in experiment 1, from 572,000 to 236,000/ml after 170 minutes in experiment 2, from 308,000 to 212,000/ml after 150 minutes in experiment 3, and from 288,000 to 114,000/ml after 170 minutes in experiment 4. It would be misleading to calculate the rates of platelet loss because the decreases were erratic. For instance, in experiment 1, the total number of platelets remained fairly steady for the first 2 hours of circulation but suddenly decreased to 50% in the next 10 minutes and then increased again.

The largest platelet aggregates were seen in experiment 2 (68-hour-old blood). These were mostly tight spherical clumps t up to 20μ in diameter and composed of 15-50 platelets. Large aggregates were found in experiment 1 (48-hour-old blood), but these were fewer in number and smaller than those seen in experiment 2. The largest aggregate found in experiment 1 consisted of 25 platelets in a loose formation (Fig. C157). In experiments 3 and 4 (3-hour-old blood), the largest aggregates consisted of 8 and 15 platelets respectively. The latter aggregate was exceptional for that experiment,

the next largest aggregate being one of 10 platelets.

Following early increases, the numbers of aggregates and the proportions of platelets involved in aggregates decreased during blood circulation in all four experiments (Figs. C158 to C162). Compared with experiments 3 and 4, the increase and decrease were much more prolonged in experiments 1 and 2. However, in experiment 4, a second mild increase in platelet aggregation occurred after 70 minutes of blood circulation.

In experiment 1, the addition of RA 233 to the blood after 25 minutes of circulation was followed by a dramatic decrease in the number of platelet aggregates commencing 35 minutes later. When fresh blood was used and two doses of RA 233 were administered in experiment 3, platelet aggregation was minimal. The use of fresh blood does not appear to have been entirely responsible for this because some aggregates were formed during experiment 4. The addition of RA 233 to the blood after 105 minutes of circulation in experiment 4 did not have a noticeable effect on platelet aggregation. A similar absence of effect was observed when the RA 233 was added to the old blood prime for experiment 2.

Doppler records obtained during experiment 4 are shown in Fig. C163. Record A in Fig. C163 was taken immediately after mixing the prime and obtaining the first blood sample. Record B was taken when the highest number of platelet aggregates was present in the blood.

Figs. C159 to C162 show rough positive relationships between the numbers of circulating platelet aggregates and the numbers of high amplitude doppler signals. These relationships were apparent only during periods of steady blood temperature. No platelet aggregates were formed during the period of steady temperature (26-28°C) in experiment 3, but for experiments 1, 2 and 4, the correlation coefficients relating the numbers of doppler echoes to the numbers of circulating platelet aggregates during periods of steady temperature are:

r_{xy} = 0.426, 0.744 and 0.652 respectively.

The corresponding regression coefficients are:

b_{yx} = 0.233, 4.620 and 4.139.

3. Gas bubbles

Materials and methods for <u>in vitro</u> experiments

The response of the ultrasonic flow detector to circulating air bubbles was tested on two occasions. While pumping saline through the circuit before priming with blood for experiment 1, 2ml of air was deliberately, rapidly introduced into the saline via the sampling tap. In experiment 4, approximately 0.5ml of air was accidentally injected through the sampling tap during blood sampling. Doppler records were obtained on both occasions.

The temperature of the circulating blood was adjusted by means of the heat exchanger in each of the four experiments described above. Rapid temperature increases were produced in experiments 1 and 3. In experiments 2 and 4, the temperature increases were more gradual.

Doppler records were obtained during these temperature changes.

After completing the tests of platelet aggregation in experiment 3 and a 1-hour delay, circulation of the blood was recommenced and rapid temperature changes were produced. At the end of experiment 4, blood circulation was continued with no time delay for 55 minutes at steady blood temperatures followed by an increase of temperature from 25°C to 36°C in 45 minutes. These experiments were numbered 3A and 4A respectively.

Results

Fig. C164 shows doppler records obtained when 2ml of air was injected into circulating saline via the sampling tap. The largest air bubbles floated to the fluid surface where they disrupted, but small air bubbles were seen to pass in an axial jet through the reservoir to be pumped into the 'arterial' tubing. High amplitude doppler responses were recorded 7 seconds after the air injection and occasional responses were still being recorded up to 1 minute after the injection.

The accidental air injection into blood also caused an increase in high amplitude doppler activity in experiment 4 (Fig. C163c). Doppler responses continued to be increased for over 5 minutes. The slower rate of bubble elimination on this occasion was presumably due to the higher viscosity of blood compared with saline.

Rapid increases in blood temperature were accompanied by increased high amplitude doppler activity in experiments 1 and 3

(Figs. C159 and C161; Table C52). More gradual temperature increases in experiments 2 and 4 did not appear to cause any increases in doppler activity (Figs. C160 and C162; Table C52). An increase in blood temperature from 26°C to 37°C in 40 minutes was accompanied by increased doppler activity in experiment 2 but the relationship was obscured by an increase in the number of circulating platelet aggregates occurring at the same time.

The effect of blood temperature changes on doppler activity in experiment 3A is shown in Fig. C165. Pronounced increases in the numbers of high amplitude doppler signals accompanied two of the three temperature increases. However, during the first temperature increase, the numbers of high amplitude doppler signals decreased. The 1-hour delay between completing the tests of platelet aggregation and recommencing blood circulating resulted in a marked increase in the numbers of high amplitude doppler signals (4-24/min). This may have been caused by cell aggregates that had formed during the circulatory stasis and perhaps they were disrupted during the next 15 minutes of blood circulation. Unfortunately, no blood samples were obtained during the second period of blood circulation.

An increase of blood temperature from 25°C to 36°C in 45 minutes during experiment 4A was accompanied by a rapid increase in doppler activity (Fig. C166). The peak for doppler activity occurred after 30 minutes of warming when the blood temperature had reached 34°C.

Considering the in vitro experiments as a whole, the changes in doppler activity associated with blood temperature increases are shown in Table C52. It appears that doppler activity can be increased by warming the circulating blood at a rate as low as 0.43°C/min when this is maintained for 15 minutes. More rapid rates of rewarming cause greater increases in doppler activity.

No bubbles were seen in the circulating blood in any of these experiments except when air was introduced through the sampling tap.

In vivo experiments

Materials and methods

Doppler records were obtained during the experiments for light- and electron-microscopy listed in Table C53. The ultrasonic flow detector was arranged and calibrated as described for in vitro experiments. Doppler records were obtained during priming, at 5-minute intervals during perfusion and at other times as required. The sampling tap was positioned with its outlet in the horizontal plane for all dog perfusions.

Results

The frequency and amplitude of doppler signals are related to the velocity of particle motion and thus to the blood flow rate (doppler effect). For this reason, it would be incorrect to compare doppler records obtained at different blood flow rates. However, there is no objection when the blood flow rate is unchanged.

Doppler records obtained during non-pulsatile dog perfusions closely resembled those obtained during in vitro blood circulation. High amplitude signals were produced during blood priming, during blood infusions into the left reservoir, when the reservoir blood level was low, during blood sampling, when solutions such as sodium bicarbonate and heparin were being injected via the sampling tap, and during blood warming (e.g. Fig. C167; effect of blood infusion).

The amplitudes of doppler signals were greater during pulsatile than during non-pulsatile perfusions at equal blood flow rates. This was expected and was undoubtedly due to the higher peak velocities of blood cells produced by pulsatile flow. However, the effects of the factors listed for non-pulsatile perfusion were also evident during pulsatile experiments (e.g. Fig. C168; effect of blood warming). Fig. C168B was recorded 2 minutes after commencing blood warming. During the first 5 minutes of this warming period, the oesophageal temperature decreased from 29.1°C to 29.0°C.

Blood samples examined before and during in vivo perfusions confirmed the in vitro observations. Red cell aggregates (rouleaux) contained in the priming blood were disrupted by circulation of the blood for a few minutes before each perfusion. Numerous large platelet aggregates were found at all stages of the perfusions and the platelet counts were severely depressed, sometimes as low as 25% during 3-hour perfusions. Microvascular filtration is presumed to be responsible for the more pronounced decreases in platelet counts

compared with in vitro experiments. These observations will be elaborated in a forthcoming thesis by Mr. R. L. Garner.

Discussion

There appears to be a relationship between the number of circulating blood platelet aggregates and the number of high amplitude doppler signals, but the correlation is not absolute. The only conclusion that can safely be drawn is that the general level of high amplitude doppler activity is related in general to the number of platelet aggregates. A more precise relationship may depend upon the development of a more accurate technique for the measurement of platelet aggregation. A screen filtration pressure apparatus has been constructed in the Keele University Workshops and it is expected that greater precision will be possible with this apparatus (Swank, 1961).

The relationship between changes in blood temperature and high amplitude doppler activity is much more pronounced. It might be argued that increased doppler activity resulted from higher peak velocities of blood cells as the blood temperature was raised. If this were so, the whole doppler flow profile would be increased in amplitude during each flow pulse.

Fig. C168 shows that this was not the case and that high amplitude doppler signals were produced at random positions in each flow pulse during blood temperature increases. A more probable explanation is that plasmatic gases were liberated as microbubbles as the gas solubility was decreased.

It must be emphasised that these experiments were intended to be exploratory rather than conclusive. Consequently, the following statements are offered as hypotheses rather than as conclusions. Further experiments are required for their verification.

1. The doppler ultrasound flow detector is a valuable instrument for investigating blood cell aggregate and microbubble formation during blood circulation.

2. Aggregates of red cells and platelets, and gas microbubbles are potential emboli in extracorporeal circuits. Fat globules have not been important in the experiments reported here.

3. Red cell aggregates form during blood storage. They are rapidly disrupted during blood circulation but form a source of emboli during blood infusions to maintain the reservoir fluid levels.

4. Platelet aggregates form during blood storage. These are more numerous and larger in old blood than in fresh blood. They are not disrupted by blood circulation. On the contrary, more aggregates are formed unless aggregation is inhibited.

5. The pyrimidopyrimidine, RA 233, may inhibit platelet aggregation and appears to potentiate the disruption of aggregates formed during blood circulation, but it has no apparent effect when added at a late stage of blood circulation, or when the blood is old. When added to 24-hour old blood at an early stage of circulation, it appears to have a delayed effect (latency of 35 minutes in experiment 3).

6. Small amounts of air introduced into the circulating blood via the sampling tap form bubbles in the reservoir. Only the largest bubbles rise immediately to the blood surface and disrupt. Smaller bubbles pass through the reservoir and are pumped into the 'arterial' tubing. In blood, several minutes of continuous circulation may be required before all of the bubbles are eliminated.

7. It is dangerous to lower the blood level below approximately 5cm from the base of the reservoir. Failure to observe this requirement results in bubble formation in the blood due to splashing.

8. The customary method of blood sampling during extracorporeal circulation is a potential hazard. If the sampling tap outlet is placed horizontally, air may be injected into the blood during sampling and during the addition of substances such as sodium bicarbonate and heparin to the blood.

9. Gas microbubbles are formed in circulating blood during rapid temperature increases. Changes in the oesophageal temperature provide a poor indication of this danger. From the <u>in vitro</u> experiments, it appeared that the safe rewarming limit is approximately $0.5^{\circ}C$/min. and that rewarming should not be maintained continuously for longer than 10 minutes. These limitations may also apply to the <u>in vivo</u> situation, but there are two reasons for not making the extrapolation at present. Firstly,

during a dog perfusion, the warmed blood enters a relatively cool vascular system, so it is possible that microbubbles may be redissolved before they cause any tissue damage. Secondly, the blood temperature was measured in the reservoir, and since the blood was observed to be inefficiently mixed in the reservoir, it follows that the recorded temperatures may not have been those of the circulating blood. During warming, the recorded temperatures may have been lower than the actual temperatures of the circulating blood. Therefore, the experimental procedure needs to be modified slightly to establish the safe limites of rewarming more precisely.

Comparison of results

The technique of extracorporeal circulation is complex. Many variations of technique are available, including the use of an oxygenator and arterial filters. Other variable factors are blood flow rate, duration of circulatory arrest, components and proportions of priming solutions, depth of hypothermia, rates of cooling and rewarming, duration of perfusion and duration of recovery. Personal skills in the performance of surgical manouevres, measurement of physiological parameters, and preparation and interpretation of histological materials add to the variation. Therefore, it would be surprising if all investigators of brain damage resulting from extracorporeal circulation had produced identical results.

The principal histological difference between the non-pulsatile (conventional) experiments reported here and previous experiments concerns the minimum insult required to produce nerve cell changes. A period of circulatory arrest has usually been thought necessary to produce brain damage of the diffuse type. Wesolowski et al (1952) subjected five dogs to 2-hour periods of cardiopulmonary by-pass using a pulsatile pump. Three dogs were sacrificed for histological examinations 5.5, 6 and 7 months later. No evidence of brain damage was found in the cerebral cortex, internal capsule, basal ganglia,

pons or cerebellum. Wesolowski's results may be criticised for his use of high blood flow rates (2 l/min. for dogs weighing 15 Kg) and his failure to prevent bronchial blood being ejected by the left ventricle. In their study of the effects upon the brain of circulatory arrest, Edmunds et al (1963) perfused one dog for 1 hour at less than 10°C and another dog for 2 hours at 37°C to provide control material. No brain damage was found in the controls. They used a mechanical piston pump with internal flap valves, but they did not describe it as a pulsatile pump and they did not provide any details of the phasic flow pulsatility.

Steegman and Miller (1965) detected minor reversible nerve cell swelling and oedema in the brains of dogs cooled to 7-8°C by the Drew (1961) technique. Periods of circulatory arrest of 30 minutes or more caused neuronal swelling and pyknosis (ACS and ICC), and astrocyte proliferation in the cerebral cortex, caudate nucleus and cerebellum. In another publication, they reported that neuronal swelling especially affected the Purkinje cells (Miller et al, 1965).

All other investigations of this type have included a period of circulatory arrest varying in duration from 15 minutes to 3 hours. Diffuse nerve cell changes were found after 15-50 minutes of circulatory arrest at 13-20°C by Schweikert and Sickinger (1960) and after 1 hour of circulatory arrest by Connolly et al (1965). The nerve cell changes consisted of ICC of Purkinje cells in the former and demyelination,

scattered nerve cell loss and glial proliferation after 2 days of recovery in the latter experiments. Focal lesions of the most advanced type described in our dogs were found in recovery animals after circulatory arrests of 15-45 minutes at 10°C by Edmunds (1964), 15-60 minutes at less than 10°C by Edmunds et al (1963) and 3 hours at 10°C by Johnston et al (1966).

The types of nerve cell change and the distribution of lesions in the brains of our dogs are in general agreement with previous reports. In light-microscope sections, the earliest detectable cell change was swelling of the perikarya of Purkinje cells (ACS). This result confirms previous reports of the selective vulnerability of the Purkinje cells in extracorporeal circulation (Almond et al, 1964; Connolly et al, 1965; Miller et al, 1965; Johnston et al, 1966).

Both diffuse and focal nerve cell changes were randomly distributed in the brain but they most frequently affected the boundary zones of the territories of the major cerebral arteries ("boundary zone" lesions of Lindenberg, 1959). In other investigations, focal lesions were also randomly distributed but the cerebral cortex, has usually been affected (Smith, 1960; Miller et al, 1962; Edmunds et al, 1963). A predilection of lesions for the boundary zones of the territories of major cerebral arteries was described by Brierley (1963; 1967).

Brierley also contrasted diffuse ("geographical") and focal

lesions in patients who had died after open-heart operations in which the Drew (1961) technique of extracorporeal circulation had been employed. However, every description of nerve cell changes in dog brains following extracorporeal circulation has included either diffuse or focal nerve cell changes, but never both. Diffuse nerve cell changes were found by Schweickert and Sickinger (1960), Almond et al (1964), Connolly et al (1965), Miller et al (1965) and Steegman and Miller (1965). Focal lesions were reported by Smith (1960), Edmunds et al (1963), Edmunds (1964) and Johnston et al (1966). In contrast, the brains of patients who had died after open-heart operations frequently contained both types of nerve cell damage (Björk and Hultquist, 1960; Silverstein et al, 1960; Fowler et al, 1962; Brierley, 1963; 1967; Javid et al, 1969). The discrepancy in results does not appear to have been due to different recovery times or to the choice of perfusion technique. No other explanation can be derived from the information available but it is probable that the choice of priming mixture, age of transfusion blood, choice of oxygenator and technique of histological fixation are relevant factors. This thesis provides the first claim that both diffuse and focal nerve cell changes can occur in the brains of dogs subjected to extracorporeal circulation and that diffuse nerve cell changes are most numerous immediately following long perfusions.

An important corrolary of the above discussion is to decide whether the neurological and psychiatric consequences of open-heart

operations correlate best with diffuse nerve cell changes, or focal lesions, or both. This is a difficult problem that is complicated by pre- and post-operative factors and by a lack of understanding of brain function. However, it is unlikely that the wide constellation of neurological and psychiatric symptoms reported could all be attributed to either diffuse or focal nerve cell changes alone. It seems more probable that transient neuroses and psychoses such as those reported by Drew (1961), Blachly and Starr (1964), Egerton and Kay (1964), Kornfeld et al (1965) and Cohen (1967) could be caused by minor, reversible diffuse nerve cell changes, while focal lesions may be responsible for epileptic seizures and asymmetrical pareses (Ehrenhaft et al, 1961; Gilman, 1965; Sachdev et al, 1967). However, no neurological sequelae were observed in our recovery dogs.

Therefore, the results of our experiments are in agreement with investigations of the neuropathological changes in human brain following open-heart surgery; they are compatible with neurological and psychiatric observations of patients; and they are complementary to previous experimental results. There have been no previous comparisons of the effects of pulsatile and non-pulsatile flows upon the brain, and no cerebral cell counts or ultrastructural studies of the brain following extracorporeal circulation.

Mechanisms of brain damage during extracorporeal circulation.
Diffuse nerve cell changes.

The principal purpose of this thesis is to examine the

possibility that brain tissue perfusion is better during pulsatile than during non-pulsatile perfusion. Experimental evidence has been provided by light- and electron-microscopical observations of dog brains following pulsatile and non-pulsatile perfusions. Diffuse nerve cell changes were associated with non-pulsatile perfusions of 2 or 3 hours duration but not with pulsatile perfusions of the same durations. Some confirmation of the light-microscopical results for non-pulsatile experiments was obtained by electron-microscopy, and in addition, subcellular changes were found immediately following 1-hour non-pulsatile perfusions.

To be thorough, it must next be shown that the differences between the brains of the pulsatile and non-pulsatile groups of dogs were not due to random or incidental physiological differences and that the possibility of fixation artefacts has been excluded.

The physiological differences were analysed in the section on 'physiological data'. The conclusions were that AP-dependent PVR was lower during pulsatile than during non-pulsatile perfusions, and that, compared with non-pulsatile perfusions, the hypotension that occurred during cannulation was more severe, venous returns were lower and consequently pump blood flow rates were lower, and $P_{a_{CO_2}}$ values were higher during pulsatile perfusions. The more severe initial hypotension and lower venous returns could not have been responsible for the absence of diffuse nerve cell changes in the pulsatile group of dogs. The lower PVR associated with pulsatile

perfusions verifies previous findings of the same kind by Nonoyama (1960), Nakayama et al (1963), Burns et al (1965), Jacobs et al (1969), Shepard and Kirklin (1969) and Trinkle et al (1969). It also fits the mathematical model of pulsatile flow dynamics presented by Shepard et al (1966) and it is compatible with the observations of microvascular changes and lymph movements made by Parsons and McMaster (1938), McMaster and Parsons (1938) and Takeda (1960).

As discussed earlier, the higher P_{aCO_2} found during pulsatile perfusions would be liable to increase cerebral blood flow (Harper, 1965; Zwetnow, 1968). However, in our experiments, the cerebral blood vessels were probably dilated by halothane anaesthesia (Christensen et al, 1967) and the effect of P_{aCO_2} may have been reduced by severe hypercapnia (Schneider, 1963; Harper, 1965) and hypotension (Zwetnow, 1968). Furthermore, tissue hypoxia causes an increase in cerebral blood flow (Pontén and Siesjo, 1965). Add to this the experimental observations that blood flow rates and arterial blood pressures were higher during non-pulsatile perfusions and the correct answer is probably that cerebral blood flow rates were higher during non-pulsatile perfusions.

Two other possible objections remain to be answered. These are that perfusion was retrograde during non-pulsatile and prograde during pulsatile experiments, and that haemolysis was presumably greater during non-pulsatile perfusions. The difference in direction of blood flow may be unimportant since it applies only to the descending aorta and not to the cerebral circulation. The effect of haemolysis is

much more important. In vitro tests have shown that the rate of haemolysis caused by the roller pump is approximately 30-times as high as that caused by the pulsatile pump. It is possible that high plasma haemoglobin levels could have caused blood sludging in the microcirculation, though there is no experimental evidence for this except that sequestered red cells were seen in some capillaries in the brains of dogs perfused with non-pulsatile flow.

No significant differences were found in an analysis of perfusion-fixation variables, but it is again noted that the collapse or obstructive occlusion of capillaries could have prevented the rapid penetration of fixative into the brain tissue and permitted post-mortem autolytic changes resembling ICC. The reversible obstructive effect of cerebral ischaemia was detected by Cantu and Ames (1969a; 1969b) and Cantu et al (1969). However, in electron-microscope experiments, larger volumes of fixative were employed and no collapsed capillaries or sequestered red cells were found, but nerve cell changes that could be correlated with the light-microscopical nerve cell changes were observed. Furthermore, in light-microscope sections, changes in the PAS reactions of nerve cells were found in regions where no collapsed capillaries or sequestered red cells were seen. Therefore, it may be that the numbers of nerve cells seen to be undergoing ICC in light-microscope sections were augmented by post-mortem autolytic changes but were not exclusively due to them.

Diffuse nerve cell changes were found in most parts of the brain following non-pulsatile perfusions. The cerebral cortex, cerebellar Purkinje cells and basal ganglia were most frequently affected. In the cerebral cortex, there was a higher incidence of both diffuse and focal nerve cell changes near the boundary zones of the major cerebral arteries. This distribution of nerve cell changes corresponds to that found after episodes of acute, profound arterial hypotension (Lindenberg, 1963; Adams et al, 1966; Brierley and Excell, 1966; Brierley et al, 1969; Brierley, 1970; Brierley et al, 1970). Profound hypoxia (Nicholson et al, 1970) and anoxia (Ingvar, 1968) produce similar distributions of lesions but the hippocampus may also be affected (Romanova and Prozorovskaya, 1966). The hippocampus is also affected by combined anoxia and ischaemia (Levine preparation) (Brown and Brierley, 1968; McGee-Russell et al, 1970). In contrast, total cerebral ischaemia primarily affects the caudate nucleus, fornix, lenticular nuclei, thalamus, pons, medulla and cerebellum, and has least effect upon the cerebral cortex (Cantu and Ames, 1969).

The earliest nerve cell change seen in our electron-microscopy experiments was a loss of neuronal and glial cytoplasm accompanied by swollen astrocyte processes and possibly swollen nerve cell perikarya. Swollen mitochondria were seen in nerve cells and astrocytes after short perfusions but they were less frequently see after long perfusions. Conversely, in some nerve cells the cytoplasmic density was increased by a proliferation of organelles, and this was

associated with the shrinkage of nerve cell perikarya and processes.

This dichotomous evolution contrasts with the sequence of events found in rat brains following combined anoxia and ischaemia (Brown and Brierley, 1968; McGee-Russell et al, 1970). In those experiments, the sequence of cell changes was microvacuolation, hyperchromatosis (ICC), ICC with incrustations, severe cell swelling and cell loss. Microvacuoles were scattered throughout the nerve cell perikarya and McGee-Russell et al (1970) concluded from electron-microscope observations of rat forebrain that the microvacuoles seen in light-microscope sections were grossly swollen mitochondria. The swollen mitochondria seen in our experiments were not sufficiently enlarged to be recognisable as microvacuoles with the light-microscope. Microvacuoles were seen close to the surface membranes of nerve cells in some toluidine blue-stained epoxy resin sections but these were clearly related to the swollen astrocyte processes and peripheral cytoplasmic vacuoles.

McGee-Russell et al (1970) claimed that reduced cytoplasmic density combined with swollen mitochondria was a fixation artefact. This possibility is not denied but there is some good experimental evidence that swelling of nerve cells, processes and organelles occurs as an early reaction to hypoxia and/or ischaemia. Edstrom and Essex (1956) concluded that the cerebral swelling that can be produced by profound anoxia is due to the intracellular accumulation of fluid. Dog kidney cortex metabolising <u>in vitro</u> increased its total tissue

water during hypoxia (Enerson, 1966). Immediately following 8 minutes of total cerebral ischaemia, the diameters of apical nerve cell dendrites in the cerebral cortex were increased by 30% (Van Harreveld, 1957). Purkinje cell dendrites and Bergmann fibres were also swollen (Van Harreveld, 1961). Chloride movements from the intertitial spaces into the dendrites were associated with the cell swelling (Van Harreveld and Schadé, 1959). Electron-microscopy of cat spinal cord fixed immediately after 50 minutes of total ischaemia disclosed a loss and clumping of synaptic vesicles, cytoplasmic swelling, local disarray of granular endoplasmic reticulum and some grossly swollen cell processes that were not identified (Van Harreveld and Khattab, 1967).

Lev et al (1965) and Keen and Dowlatshahi (1970) used electron-microscopy to identify intracellular swelling of myocardial cells during extracorporeal circulation. The latter authors also found disrupted mitochondria and aggregated nuclear proteins during the period of circulatory arrest.

A multitude of physiological and chemical treatments are known to cause mitochondrial swelling (Rouiller, 1960). Mitochondrial and other organelle swelling has been found after several experimental procedures liable to produce hypoxia and/or ischaemia (Hager, 1963; Webster and Ames, 1965; Bakay and Lee, 1968; Lee et al, 1968; Miyagishi and Suwa, 1969; Hauschild et al, 1970; Williams and Grossman, 1970). Webster and Ames (1965) found that the progressive swelling of mitochondria, Golgi membranes and granular endoplasmic

reticulum and the loss of synaptic vesicles caused by combined oxygen and glucose deprivation were completely reversible if the insult was not continued for longer than 20 minutes. Packer (1963) discriminated between normal, low amplitude swelling and abnormal, high amplitude swelling of mitochondria. Highly active mitochondria shrink but decreased respiratory rates cause mitochondria to swell by as much as 40%.

Only Jennings et al (1967) were unable to find any abnormal mitochondria following ischaemic injury. However, they did find that the oxidative and phosphory lative capacities of myocardial mitochondria were reduced to about 40% of the control values by 15 minutes of total ischaemia, and little metabolic activity remained after 2 hours of total ischaemia. Losses of respiratory enzymes were found in the brains of rats exposed to pure nitrogen atmospheres for 30 minutes by MacDonald and Spector (1963) and by Yap and Spector (1965).

The formation of peripheral clear spaces in some nerve cells of our dogs may be considered to be a sign of either cell swelling, or cytoplasmic shrinkage, or simply a loss of organelles. The shapes of nerve cells with peripheral clear spaces suggested that the cells might be slightly swollen. Astrocytes with reduced numbers of organelles were more definitely swollen.

Therefore, only Brown and Brierley (1968) and their associates

(McGee-Russell et al, 1970) have found swollen mitochondria associated with cell shrinkage. The majority opinion appears to be that swelling of cells, cell processes and organelles is associated with a loss of cytoplasmic organelles and that both swollen mitochondria and swollen cells can be produced by hypoxia and/or ischaemia.

In light-microscope sections, it is difficult if not impossible to distinguish between early ischaemic cell changes and 'dark cell' fixation artefacts. Dark cells have generally been recognised as a consequence of immersion- or inadequate perfusion-fixation (Koenig et al, 1945; Koenig and Koenig, 1952; Cammermeyer, 1960a; 1960b; 1962; Friede, 1963; Wright and Sanderson, 1970). Nemetschek-Gansler and Becker (1964) thought that dark cells should not be considered as artefacts since they represent a genuine pathological state of nerve cells undergoing post-mortem autolysis. This view was supported by Westrum and Lund (1966) and by Kvitnitskii-Ryzhov and Pelipas (1968).

However, the ultrastructural changes found during the early stages of post-mortem autolysis could not be expected to be represented by hyperchromatic cells in light-microscope sections. In electron-micrographs of rat brains allowed to undergo post-mortem autolysis before fixation, Karlsson and Schultz (1966) found widened cisternae and stacking of α-cytomembranes (endoplasmic reticulum), curved Golgi complexes, clumped nuclear material, finely granular amorphous electron-opaque material especially in Purkinje cell dendrites, and decreased numbers of synaptic vesicles, the remainder of which were flattened.

Van Nimwegen and Sheldon (1966) found rounded Golgi complexes, lamellar bodies in the endoplasmic reticulum, clumped nuclear chromatin, and some dumbbell and ring-shaped mitochondria in similar preparations.

The 'dark cells' of electron-microscopy are so-called because of their high electron-density. Dark nerve cells and oligodendrocytes found in the brain of Myxine had crenated outlines, swollen processes and a high electron-density of the cytoplasm (Mugnaini, 1965). These were considered to be fixation artefacts because their frequency appeared to be dependent upon the tonicity of the fixative. Dark nerve cells and processes were identified in electron-micrographs of the normal ventrobasal thalamic nucleus of cats (Cohen and Pappas, 1969). The cells stained deeply with toluidine blue in epoxy-resin sections and the electron-micrograph in their publication showed distorted cell membranes, increased electron-density of the cytoplasm and expanded Golgi cisternae.

Comparison of the ultrastructural changes in the 'dark' nerve cells of our dogs with those previously reported to be fixation artefacts reveals a poor correspondence. In the dogs, the increased cytoplasmic density was due to a proliferation of organelles particularly endoplasmic reticulum and free ribosomal rosettes. No organelles appeared to be swollen. In artefactual changes the increased cytoplasmic density is due to a finely granular amorphous material that is evenly distributed throughout the cytoplasm.

Organelles are often swollen.

Many experimenters have demonstrated increases in the numbers of cytoplasmic organelles in response to cellular injury. In their Levine preparations, McGee-Russell et al (1970) found increases in cytoplasmic density in nerve cells of the forebrain. They were associated with aggregates of ribosomes and ribosomal rosettes, swollen mitochondria, tubules, vesicles and cisternae of the endoplasmic reticulum, aggregated nuclear material and widened intra-membrane nuclear spaces. There were no increased numbers of dense bodies, Golgi complexes or granular endoplasmic reticulum.

Barron et al (1970) found peripheral displacements of Golgi complexes and a proliferation of subsurface cisternae (endoplasmic reticulum) in cervical cord chromatolytic nerve cells 15 days after brachial plexectomy in cats. Dixon (1969) reported increased amounts of granular and agranular endoplasmic reticulum in the satellite cells of chromatolytic neurons 3-168 days after axotomy. Van Breeman and Bowman (1966) thought the complex stacks of endoplasmic reticulum found in dog cerebellar Purkinje cells were due to hypoxia incurred during the process of preparation for electron-microscopy. This was a report on one dog subjected to decompression to 1mm Hg for 2 minutes.

Increased numbers of lysosomes (dense bodies) have been found in rat brain immediately after repeated episodes of combined anoxia and ischaemia, and after 10 minutes of post-mortem autolysis (Becker and Barron, 1961). Lysosomes were increased both in size and numbers

in cat motoneurons 30-60 minutes after 50 minutes of spinal cord asphyxiation (Khattab, 1967).

According to the theory of Weiss (1967), lysosomal enzymes may be released from their ionic bonds into the cytoplasm in response to cell injury. The reaction may take the form of total destruction of the cell or of a chronic "weeping lesion" in which lysosomal enzymes are continually resynthesised and from which the cell may recover. Thakar and Tewani (1967) took a different view. They described four types of lysosome arrangement based upon acid phosphatase studies of the nerve cells of bat cerebellum, spinal cord and trigeminal ganglia. In their view, vesicular and granular arrangements of acid phosphatase (lysosomes) are normal. A perinuclear distribution occurs as an early response to cell injury and represents an early intensification of protein synthesis culminating in a diffuse intracellular distribution of acid phosphatase. Two stages in the evolution of reactive lysosomes were described by Dianzani et al (1969). In stage 1 (primary lysosomes), the lysosomes are devoid of histochemically detectable acid phosphatase and they are negative to the diastase-PAS reaction, but in stage 2 (secondary lysosomes), they are positive to both tests.

Unidentified PAS-positive granules were found in most normal nerve cells in our dogs and the reaction became diffuse in early ICC. This contrasts with the experimental findings of Steegman and Miller

(1965). They did not find PAS-positive granules in dog brain fixed by Cammermeyer's (1962) technique, but PAS-positive granules were present in the blood vessel walls and in the perivascular spaces of dogs surviving for several days after extracorporeal circulation. Diffuse PAS-positive reactions were found in the ground substance of the grey matter and in occasional nerve cells. Sulkin (1955) was also unable to detect PAS-positive granules in the nerve cells of young dogs but they were present in senile dogs. Using more specific histochemical tests, he detected mucoproteins in the nerve cells of both young and old dogs and concluded that the concentrations of mucoproteins in young dog brain were too low to be detectable by the PAS technique.

Thus, it is possible that the increase in cytoplasmic materials found in the nerve cells of dogs subjected to extracorporeal circulation was a result of increased protein synthesis and the diffuse PAS-positive reaction could be due to the release of lysosomal enzymes, and/or the exposure of 1,2 glycol groups during autolysis, and/or the conversion of primary to secondary lysosomes, and/or the production of PAS-positive proteins such as mucoproteins.

The increased concentrations of endoplasmic reticulum and ribosomal rosettes and the more irregular outlines of the nuclear membranes would seem to support the hypothesis that protein synthesis is enhanced. This would require higher rates of protein synthesis from neuronal ribonucleic acid (RNA) or increased amounts of RNA.

The former has been found during stagnant hypoxia of 12-day-old rats (Sirakova et al, 1968) and the latter during anoxia of adult rats (Pevzner, 1968). It is known that macromolecules such as RNA can be transported from glial to neuronal cytoplasm (Pevzner, 1968) and this may be an early stage of the reaction followed by the construction of more endoplasmic reticulum.

Thus, there is some confusion about the causes of increases and decreases in cytoplasmic density resulting from experimental procedures involving hypoxia and/or ischaemia. It seems most likely that the primary intracellular response is from the lysosomes. The liberation of lysosomal enzymes may then result in the katabolism of cytoplasmic components. Reduced rates of respiration would then permit the free entry of diffusible ions and water into the cell cytoplasm and its organelles, and this process could not be reversed by higher rates of active transport. Further cytoplasmic damage would result from overhydration (positive feedback). Alternatively, the liberation of lysosomal enzymes may catalyse protein synthesis if reserve synthetic capacity is available. This would be accomplished by the glial to neuronal transfer of RNA and increased template activity of RNA. Increased rates of respiration would be required from the mitochondria and they would adopt their contracted form. Cell shrinkage could be a consequence of the increased oncotic pressure of the extracellular fluid resulting from inadequate lymph movement

as explained below and the increased synthesis of protein may be the cell's attempt to reduce the oncotic gradient. Eventually cellular respiration would be limited by oxygen availability (negative feedback).

According to this theory, whether a nerve cell swells or shrinks in response to hypoxic and/or ischaemic injury will depend upon its ability to maintain the selective permeability of the surface membrane. The factors involved will be the rate of induction, severity and duration of the insult, the availability of nutrient reserves, the previous physiological state of the cell and the amount of reserve synthetic capacity. Therefore, the cell's reaction to hypoxic and/or ischaemic insult will be determined by a balance of factors and cell swelling may be converted to cell shrinkage, or vice versa, by a redistribution of the balance. Alternatively, the cell may recover if the insult is not prolonged and if the cell is not too severely damaged.

This theory differs from previous ones in its inclusion of an active stage of cellular reaction during shrinkage, changes in cytoplasmic density preceding cell swelling or shrinkage, and a mechanism by which both swelling and shrinkage can be produced by similar insults (see review by Cammermeyer, 1962). The results of Sirakova et al (1968) support the hypothesis of a dichotomous mechanism. They found that as a result of hypoxia, the nerve cells of young rats developed cytoplasmic vacuoles, whereas in adult rats they

became pyknotic. Conversely, anoxia caused vacuolation in adults and pyknosis in young rats.

Bearing in mind the possible effects of haemolysis on the microcirculation, the initial cell insult in our experiments was probably hypoxic ischaemia resulting from non-pulsatile blood flow. Its effect upon the nerve cells would not be expected to be the same as that caused by ligating a carotid artery. The mechanism may be as follows:- The formation and flow of lymph are dependent upon mechanical agitation of the tissues by arterial pulsations. In the absence of these pulsations, tissue fluid will accumulate and the interstitial fluid pressure will rise. The pressure gradient between the capillaries and the extracellular spaces will be decreased. However, tissue fluid will continue to accumulate due to the failure of the lymphatics to remove large molecules from the extracellular spaces. Capillary blood flow will decrease due to the increasing tissue fluid pressure upon the capillary walls, and eventually some capillaries may collapse. This would cause the PVR to rise and the expected histological result would be scattered regions of ACS and/or ICC associated with collapsed capillaries and sequestered blood cells.

Therefore, the experimental results can be incorporated into a theory of diffuse nerve cell reaction to hypoxic and/or ischaemic insult and the insult can be explained by a theory of non-pulsatile flow.

No nerve cell damage was seen by light-microscopy immediately after 1-hour of non-pulsatile perfusion, but it was visible in electron-micrographs. Furthermore, the brains of animals that lived for a few hours following 1-hour non-pulsatile perfusions contained some diffuse nerve cell changes and there were signs of generalised responses such as laminar astrocyte proliferation and increased numbers of Maltese cross phagocytes in the subarachnoid spaces. The origin of these phagocytes is not known and their anisotropy did not appear to be due to the incorporation of myelin lipids because no myelin degeneration was observed.

The cerebral cell counts were increased after all experimental procedures, but it was not clear whether this was entirely due to cell swelling or whether glial proliferation and inflitration could have played some part.

Electron-microscope studies showed that both increases and reductions of cytoplasmic materials followed 3 hours of anaesthesia with the thorax open. These changes may be attributed to hypotensive ischaemia. The few nerve cells with increased cytoplasmic densities were found in dog 148. As shown in Fig. C20, the AP declined more rapidly in experiment 148 than in other experiments of the same type. After 3 hours, the AP had reached 44mm Hg in experiment 148, 52mm Hg in experiment 146 and 60mm Hg in experiment 153. These AP values are close to the critical value of 50mm Hg, below which cerebral blood flow autoregulation is abolished (Zwetnow, 1968). Hypotension is

induced by halothane anaesthesia (Raventós, 1956) and cardiac output is reduced by lowering the body temperature (Shumacker, 1960). On the other hand, both halothane and nitrous oxide depress the cerebral metabolic consumption of oxygen (Theye and Michenfelder, 1968a; 1968b). Finally, barbiturates such as sodium thiopentone are known to have a histotoxic anoxic effect on the brain (Brazier and Finesinger, 1945; Homburger et al, 1946), so the analysis of cause and effect is too complicated to be resolved.

Focal lesions

All but one of the recovery animals had developed focal lesions in the brain. The exception was perfused with blood collected on the morning of the experiment. One focal lesion was detected in the brain of dog 144 which was also perfused with fresh blood. All of the other dogs had been perfused with blood collected 24-48 hours earlier. It is probable that the age of the blood used to prime the circuit is a determinant factor in cerebral embolism.

Aggregates of red cells and platelets were found in stored blood used to prime the circuit for in vitro experiments. These were larger and more numerous in old blood compared with fresh blood. Red cell aggregates may be important when blood is infused into the reservoirs during dog perfusions. Platelet aggregation increases during blood circulation and is probably a major cause of cerebral embolic lesions.

Platelet aggregates were found in stored blood by Swank (1961) Swank and Porter (1963) and Hirsch et al (1964). Platelet emboli have been identified as eosinphilic, granular masses in the heart, lungs and kidneys of patients who died after open-heart operations (Jenevein and Weiss, 1964; Ashmore et al, 1968). They have not previously been found in the brain and none were found in our dog brains. Eosinphilic, granular masses were certainly present in the lungs of some animals but they could not definitely be identified as platelet aggregates. It is possible that the platelet aggregates may have been removed from the arteries after they had caused some ischaemic necrosis. Swank and Porter (1963) claimed that platelet aggregates are removed by the reticuloendothelial system. Rumbaugh et al (1969) found that autologous emboli injected into dog carotid arteries tend to lodge at the bifurcations of arteries but during the first 24 hours after injection, they fragment and move into smaller arteries and arterioles. By the end of the first 24 hours after injection, they found that the emboli were either absent or only just detectable in angiographs.

Platelet aggregates have been found during extracorporeal circulation by Swank and Porter (1963), Swank et al (1963), Ashmore et al (1968) and Patterson and Kessler (1969). Jenevein and Weiss (1964) found them during massive blood transfusions. Filtration of the blood through synthetic wool has been recommended for their removal (Swank, 1961; Hirsch et al, 1964). In my experience, this

procedure causes severe reductions in platelet numbers. A more satisfactory solution would be to inhibit platelet aggregation with pyrimidopyrimidine compounds (Emmons et al, 1965; Didisheim and Owen, 1970; Didisheim et al, 1970).

Gas microbubbles were detected in circulating blood during in vitro experiments and it is possible that microbubbles could have been responsible for some focal lesions during dog perfusions. Further experiments are required to establish the conditions for microbubble formation in circulating blood.

Gas bubbles were found in the blood during extracorporeal circulation by Jordan et al (1958), Selman et al (1967) and Aronstam et al (1968). Factors thought to be responsible for the formation of gas bubbles were high oxygen tensions in the oxygenator, high flow rates of oxygen into the oxygenator, prolonged recirculation of physiological saline before priming pump-oxygenator circuits with blood, agitation of the oxygenator, turbulent blood flow, rapid blood temperature changes (Donald and Fellows, 1959) and pressure fluctuations caused by roller pumps (Bass and Longmore, 1969).

The importance of turbulence and agitation of the oxygenator in pump-oxygenator techniques may have been under-estimated. Chistoforides and Hedley-Whyte (1969) showed the effect of fluid motion upon gas solubility in vitro. They equilibrated human blood with oxygen at 760mm Hg partial pressure at 2°C. The tension was increased to 1400mm Hg by raising the blood temperature to 37°C, but stirring the blood then caused it to fall rapidly back to 760mm Hg

showing that oxygen is rapidly released from solution by the combination of raised temperature and turbulence.

The use of an ultrasound flow detector for identifying microbubbles (Maroon et al, 1969; Patterson and Kessler, 1969) and particulate matter (Austen and Howry, 1965) appears to be a valuable adjunct for extracorporeal circulation. The experiments reported in the previous section of this thesis show that doppler activity is influenced by microbubbles and probably by platelet aggregates in the circulating blood. Consultation of the scientific literature shows that both microbubbles and platelet aggregates could be responsible for focal brain lesions.

Prospective work

The work completed to date has created more problems than it has solved. It may be considered that the case for using pulsatile blood flow in extracorporeal circulation is established but the influence of incidental factors such as high P_{aCO_2}, haemolysis and retrograde perfusion needs to be clarified.

In some forthcoming experiments, it is proposed to use a pump-oxygenator system. Since the lungs will not be perfused, it is hoped that P_{aCO_2} will be easier to control than in the whole-body perfusion technique. Retrograde perfusion will be abandoned in favour of prograde perfusion via the proximal aorta for non-pulsatile perfusions. Whole-blood priming will also be replaced by priming with a mixture

of Hartmann's solution and low molecular weight dextran. This should reduce the effect of haemolysis on the microcirculation, if any such effect exists.

The pulsatile pump does not provide a consistent flow output and does not incorporate adequate safety precautions to make it usable for open-heart operations. Further developments of this pump and a new mechanically activated pulsatile pump are in progress.

Further histochemical investigations are required to establish the identity of the cytoplasmic materials accumulating in nerve cells undergoing early ICC. In particular, an attempt will be made to identify the PAS-positive substances.

A further undertaking will be to develop a pulse damping device that can be applied to the cerebral circulation unilaterally. With this simpler experimental system the problems of eliminating fixation artefacts and of the time course of reversible nerve cell changes resulting from non-pulsatile flow may be studied more conveniently.

Immediate plans concern the elimination of cerebral emboli during extracorporeal circulation. A series of experiments has been planned to compare the incidence of focal lesions in the brains of dogs perfused with a pulsatile pump-oxygenator system when the pyrimidopyrimidine compound RA 233 is included and omitted from the prime. Great care will be taken during these experiments to ensure that air emboli are not created. Some suggestions for their

prevention are made below.

Finally, the in vitro studies of platelet aggregation and microbubble formation will be completed.

Conclusions and recommendations

The main conclusions of this work are as follows:-

1. By employing a new technique of perfusion-fixation (Wright and Sanderson, 1970), discrimination between normal nerve cells and nerve cells undergoing early ischaemic cell changes was facilitated.
2. Non-pulsatile perfusion of dogs produced diffuse nerve cell changes that were detectable by electron-microscopy immediately after 1-hour perfusions and by light-microscopy after 2 and 3-hour perfusions.
3. No diffuse nerve cell changes were found in the brains of dogs subjected to 2 and 3-hour pulsatile perfusions.
4. Early ischaemic cell change may be identified by a diffuse intracellular PAS-positive reaction in light-microscope sections and by increased numbers of cytoplasmic organelles in electron-micrographs.
5. A loss of cytoplasmic materials, possibly associated with nerve cell and astrocyte swelling may be an early response to ischaemic injury but these changes can also occur as fixation artefacts.

6. Diffuse nerve cell changes may be reversible.

7. Focal lesions may be found in the brains of dogs as early as immediately after 2-hour perfusions, and they are usually prominent when the animals are allowed to survive for a few days.

8. Focal lesions may be attributed to embolism by aggregates of blood cells and gas microbubbles.

To reduce the incidence of brain damage resulting from extracorporeal circulation both in dogs and in human patients, the following recommendations are made:-

1. A pulsatile pump should be employed as soon as a reliable and safe one becomes available.

2. If blood is used for priming the circuit or for infusion into the circuit during perfusion, it should be as fresh as possible.

3. Modification of the design of existing blood reservoirs should be undertaken to reduce splashing and to restrict the passage of gas bubbles through the reservoir.

4. The fluid meniscus in the reservoir should never fall below the level of the inlet opening.

5. The blood sampling tap should be positioned with its outlet in the vertical plane.

6. Solutions for injection into the circulating blood via the sampling tap should be free from gas bubbles.

7. Further experimental work is required to develop a reliable and safe pulsatile pump, to establish more precisely the safe limits of blood warming, and to determine the value of chemical inhibitors of platelet aggregation during extra-corporeal circulation.

APPENDIX 1

THE MORTON-KEELE PULSATILE PUMP

The following account is derived and modified from a description of the Morton-Keele pulsatile pump by Mr. P. Morton of Stafford Estate Laboratories, General Electric Company Power Engineering Limited, Stafford (G.E.C.). The design is covered by international patents but it has not yet been published.

Principles of the pump

The pump (Fig. D1) was designed primarily to be suitable for use in open-heart surgery on human patients. Hydraulic power is used to actuate the alternative types of pump head. Fig. D2 shows the pump with ventricle (A, G.E.C. prototype) and diaphragm (B, Harvard Apparatus Sales Corporation, model 1413) pump heads. The Harvard head was used for all of the pulsatile experiments reported in this thesis. It is constructed with perspex and incorporates a silastic rubber diaphragm to divide the pump head into water and blood compartments. Ball valves regulate the direction of blood flow to and from the blood chamber.

Water flow into and out of the water chamber of the pump head is controlled by a simple, reciprocating two-land spool valve that connects the pump head alternately to 0.5 horse power centrifugal pressure (PP) and suction (SP) pumps. The valve is submerged in

water in a reservoir fed from the pressure pump and supplying the suction pump, so that spool valve leakage is of no importance.

Stroke volume is continuously variable from 0-100 ml/stroke by offsetting the valve body and thus varying the duration of connection between the pressure line and the diaphragm. Stroke frequency is controlled from 0-200 strokes/min. by a variable speed motor driving a crank that actuates the spool. Pumping water against a 244 cm (8 feet) pressure head, the maximum flow rate obtained was 4.1 l/min. at 100 strokes/min.

For total cardiac bypass, two pump heads are connected to two spool valves assembled adjacent to each other. The pump heads share the same source of power, water circuit and frequency control, but stroke volume is separately controlled via each spool valve.

APPENDIX 2

THE PERFUSION-FIXATION TECHNIQUE

Abram, H.S. Adaptation to open-heart surgery: A psychiatric study of response to the threat of death. Am. J. Psychiat., 122, 659-668, 1965.

Adams, J.E., Owens, G., Mann, G., Headrick, J.R., Munoz, A., and Scott, H.W. Experimental evaluation of Pluronic F-68 (a non-ionic detergent) as a method of diminishing systemic fat emboli resulting from prolonged cardiopulmonary bypass. Surg. Forum, 10, 585-589, 1959.

Adams, J.H., Brierley, J.B., Connor, R.C.R. and Treip, C.S. The effects of systemic hypotension upon the human brain, clinical and neuropathological observations in II cases. Brain 89, 235-268, 1966.

Adolph, R. J., Fukusumi, H. and Fowler, N.O. Origin of cerebrospinal fluid pulsations. Am. J. Physiol., 212, 840-846, 1967.

Allen, J.G. Extracorporeal circulation. Springfield, Illinois, Thomas, 518pp, 1958.

Allen, P. Central nervous system emboli in open heart surgery. Can. J. Surg., 6, 332-337, 1963.

Almond, C.H., Jones, J.C., Snyder, H.M., Grant, S.M. and Meyer, B.W. Cooling gradients and brain damage with deep hypothermia. J. thorac. cardiovasc. Surg., 48, 890-897, 1964.

Anabtawi, I.N., Womack, C.E. and Ellison, R.G. Thoracic duct lymph flow during pulsatile and nonpulsatile extracorporeal circulation. Ann. thorac. Surg., 2, 38-43, 1966.

Anderson, M.N. and Senning, Å. Studies in oxygen consumption during extracorporeal circulation with a pump-oxygenator. Ann. Surg., 148, 59-65, 1958.

Anderson, R.M., Fritz, J.M. and O'Hare, J.E. The mechanical nature of the heart as a pump. Am. Heart J., 73, 92-105, 1967.

Ankeney, J.L. and Viles, P.H. The effect of total body perfusion on carotid blood flow. Surgery, St. Louis, 49, 209-214, 1961.

Arfel, G., Casanova, C., Naquet, R., Passelq, J. and Dubost, C. Étude électro-clinique de l'embolie gazeuse cérébrale en chirurgie cardiaque. Electroenceph. clin. Neurophysiol., 23, 101-122, 1967.

Aronstam, E.M., Geiger, J.P., Morris, J.A. and Rigby, P.T. Preliminary observations on the performance of the Bramson membrane lung oxygenator. Ann. thorac. Surg., 5, 367-370, 1968.

Ashmore, P.G., Svitek, V. and Ambrose, P. The incidence and effects of particulate aggregation and microembolism in pump-oxygenator systems. J. thorac. cardiovasc. Surg., 55, 691-697, 1968.

Astrup, P.,Jørgensen, K., Siggaard Andersen, O. and Engel, K. The acid base metabolism. A new approach. Lancet, vol. I (1960), 1035-1039.

Attinger, E.O. Flow patterns and vascular geometry. In: Pulsatile blood flow (E.O. Attinger, ed.). New York, Toronto and London, McGraw-Hill, pp 179-200, 1964.

Austen, W.G. and Howry, D.H. Ultrasound as a method to detect bubbles or particulate matter in the arterial line during cardiopulmonary bypass. J. Surg. Res, 5, 283-284, 1965.

Bailey, N.T.J. Statistical methods in biology. London, E.U.P., 200 pp, 1959.

Barron, K.D., Daniels, A.C., Chiang, T.Y. and Doolin, P.F. Fine structure of chromatolytic feline motoneurons. Exp. molec. Path., 12, 46-57, 1970.

Bass, R.M. and Longmore, D.B. Cerebral damage during open heart surgery. Nature, Lond., 222, 30-33, 1969.

Baue, A.E., Nusbaum, M., Anstadt, F. and Blakemore, W.S. The pattern of lymphatic flow during extracorporeal circulation. J. thorac. cardiovasc. Surg., 50, 648-657, 1965.

Baust, G., Paver, H.-D. and Fischer, U. Blutfluss und Pulsform im extrakörporalen kreislauf. Thoraxchirurgie, 16, 184-189, 1968.

Becker, N.H. and Barron, K.D. The cytochemistry of anoxic and anoxic-ischemic encephalopathy in rats. I. Alterations in neuronal lysosomes identified by acid phosphatase activity. Am. J. Path., 38, 161-175, 1961.

Bennett, P.N. Role of erythrocytes in the acute platelet response to operation. J. clin. Path., 21, 695-697, 1968.

Bering, E.A. Choroid plexus and arterial pulsation of cerebrospinal fluid. Archs Neurol. Psychiat., Chicago, 73, 165-172, 1955.

Björk, V.O. and Hultquist, G. Brain damage in children after deep hypothermia for open-heart surgery. Thorax, 15, 284-291, 1960.

Blachly, P.H. and Kloster, F.E. Relation of cardiac output to post-cardiotomy delirium. J. thorac. cardiovasc. Surg., 52, 422-427, 1966.

Blachly, P.H. and Starr, A. Post-cardiotomy delirium. Am. J. Psychiat., 121, 371-375, 1964.

Blackwood, W. Vascular disease of the central nervous system. In: Greenfield's neuropathology (W. Blackwood, W.H. McMenemey, A. Meyer, R.M. Norman and D.S. Russell, eds.), 2nd edit. London Arnold, pp71-137, 1963.

Bleifield, W. Cerebrale "fett" embolie nach Operationen mit der Herz-Lungen-Maschine. Thoraxchirurgie, 9, 12-19, 1961.

Bok, S.T. Histonomy of the cerebral cortex. Amsterdam, New York and Princeton, Elsevier, pp8-59 and 60-82, 1959.

Brazier, M.A.B. and Finesinger, J.E. Action of barbiturates on the cerebral cortex. Archs Neurol. Psychiat., Chicago, 53, 51-58, 1945.

Brierley, J.B. Neuropathological findings in patients dying after open-heart surgery, Thorax, 18, 291-304, 1963.

Brierley, J.B. Cerebral injury following cardiac operations. Lancet, vol. I (1964), 175-176.

Brierley, J.B. Brain damage complicating open-heart surgery: A neuropathological study of 46 patients. Proc. R. Soc. Med., 60, 858-859, 1967.

Brierley, J.B. Systemic hypotension - neurological and neuropathological aspects. Modern trends in neurology, vol. 5. London, Butterworth, pp164-177, 1970.

Brierley, J.B., Brown, A. W., Excell, B.J. and Meldrum, B.S. Brain damage in the rhesus monkey resulting from profound arterial hypotension. I. Its nature, distribution and general physiological correlates. Brain Res., 13, 68-100, 1969.

Brierley, J.B., Brown, A.W.,Meldrum, B.S. and Riche, D. The time course of ischaemic neuronal changes in the primate brain following profound arterial hypotension, air embolism and hypoglycaemia. J. Physiol. Lond., 207, 59P, 1970.

Brierley, J.B. and Excell, B.J. The effects of profound systemic hypotension upon the brain of M. rhesus: Physiological and pathological observations. Brain, 89, 269-298, 1966.

Brown, A.H., Braimbridge, M.V., Niles, N.R., Gerbode, F. and Aguilar, M.J., The effect of excessively high perfusion pressures on the histology, histochemistry, birefringence, and function of the myocardium. J. thorac. cardiovasc. Surg., 58, 655-663, 1969.

Brown, A.W. and Brierley, J.B. The nature, distribution and earliest stages of anoxic-ischaemic nerve cell damage in the rat brain as defined by the optical microscope. Br. J. exp. Path., 49, 87-106, 1968.

Burns, W.H., McInnes, A.G.P. and Mandelbaum, I. Hemodynamic studies with a new pulsatile pump. Trans. Am. Soc. artif. internal Organs, 11, 65-67, 1965.

Burton, A.C. Relation of structure to function of the tissues of the wall of blood vessels. Physiol. Rev., 34, 619-642, 1954.

Caguin, F. and Carter, M.G. Fat embolism with cardiotomy with the use of cardiopulmonary bypass. J. thorac. cardiovasc. Surg., 46, 665-672, 1963.

Cammermeyer, J. A critique of neuronal hyperchromatosis. J. Neuropath. exp. Neurol., 19, 141-142, 1960a.

Cammermeyer, J. The post-mortem origin and mechanism of neuronal hyperchromatosis and nuclearpyknosis. Expl. Neurol., 2, 379-405, 1960b.

Cammermeyer, J. An evaluation of the significance of the "dark" neuron. Ergebn. Anat. EntwGwsh., 36, 1-61, 1962.

Cantu, R.C. and Ames, A.A. Distribution of vascular lesions caused by cerebral ischemia. Neurology, Minneap., 19, 128-132, 1969a.

Cantu, R.C. and Ames, A.A. Experimental prevention of cerebral vasculature obstruction produced by ischemia. J. Neurosurg., 30, 50-54, 1969b.

Cantu, R.C., Ames, A., Dixon, J. and Digiacinto, G. Reversibility of experimental cerebrovascular obstruction by complete ischemia. J. Neurosurg., 31, 429-431, 1969.

Černý, J., Somogyi, J. and Rudolf, V. Unmittelbare Veranderungen der Hirnrindenstruktur wärend Hypothermie und erwarmung. Zentbl. allg. Path. path. Anat., 108, 579-584, 1966.

Christensen, M.S., Høedt-Rasmussen, K. and Lassen, N.A. Cerebral vasodilation by halothane anaesthesia in man and its potentiation by hypotension and hypercapnia. Br. J. Anaesth., 39, 927-934, 1967.

Christoforides, C. and Hedley-Whyte, J. Supersaturation of blood with O_2. J. Appl. Physiol., 26, 239-240, 1969.

Clarke, C.P., Kahn, D.R., Dufek, J.H. and Sloan, H. The effects of nonpulsatile blood flow on canine lungs. Ann. thorac. Surg., 6, 450-457, 1968.

Clowes, G.H.A. Extracorporeal maintenance of circulation and respiration. Physiol. Rev., 40, 826-919, 1960.

Cohen, E.B. and Pappas, G.D. Dark profiles in the apparently-normal central nervous system: A problem in the electron microscopic identification of early antero-grade axonal degeneration. J. comp. Neurol., 136, 375-395, 1969.

Cohen, S.I. Neurological and psychiatric aspects of open-heart surgery. Thorax, 19, 575-578, 1964.

Cohen, S.I. Neurological complications of cardiac surgery and respiratory disorders: Some psychiatric aspects. Proc. R. Soc. Med., 60, 859-860, 1967.

Connelly, J.E., Roy, A., Guernsey, J.M. and Stemmer, E.A. Bloodless surgery by means of profound hypothermia and circulatory arrest. Effect on brain and heart. Ann. Surg., 162, 724-737, 1965.

Dalton, M.L., McCarty, R.T., Woodward, K.E. and Barila, T.G. The army artifical heart pump. II. Comparison of pulsatile and nonpulsatile flow. Surgery, St. Louis, 58, 840-845, 1965a.

Dalton, M.L., Mosley, E.C., Woodward, K.E. and Barila, T.G. The effect of pulsatile flow on renal blood flow during extracorporeal circulation. J. Surg. Res., 5, 127-131, 1965b.

Danielson, G.K., Dubilier, L.D. and Bryant, L.R. Use of Pluronic F-68 to diminish fat emboli and hemolysis during cardiopulmonary bypass. J. thorac. cardiovasc. Surg., 59, 178-184, 1970.

Dardenne, G., Dereymaeker, A. and Lacheron, J.M. Cerebrospinal fluid pressure and pulsatility. An experimental study of circulatory and respiratory influences in normal and hydrocephalic dogs. Eur. Neurol., 2, 193-216, 1969.

DeBakey, M.E. Simple continuous-flow blood transfusion instrument. New Orl. med. surg. J., 87, 386-389, 1934.

De Langen, C.D. Blood pressure, pulse pressure and tissue flow. Cardiologia, 33, 249-258, 1958.

De Langen, C.D. Die klinische Bedeutung der Pulsdruckamplitude. Münch. med. Wschr., 101, 2132-2135, 1959.

Dennis, C. Certain methods for artifical support of the circulation during open intracardiac surgery. Surg. Clins N. Am., New York number, April issue. Philadelphia and London, Saunders, pp423-436, 1956.

Dennis, C., Spreng, D.S., Nelson, G.E., Karlson, K.E., Nelson, R.M., Thomas, J.V., Eder, W.P. and Varco, R.L. Development of a pump oxygenator to replace the heart and lungs; an apparatus applicable to human patients and application to one case. Ann. Surg., 134, 709-721, 1951.

Dianzani, M.U., Baccino, F.M. and Rita, G.A. The vacuolar degeneration of cells (congestive enlargement of lysosomes). Acta anat., suppl. 56, vol. 73, 152-181, 1969.

Didisheim, P., Bowie, E.J.W. and Owen, C.A. Inhibition of arterial thrombosis and platelet adhesiveness in the rat by intravenous 2,4,6-trimorpholinopyrimido-(5,4-d)-pyrimidine (RA 433). Mayo Clin. Proc. 45, 51-59, 1970.

Didisheim, P. and Owen, C.A. Prevention of experimental arterial thrombosis and platelet adhesiveness by 2,6-bis-(diethanolamino)-4-piperidinopyrimido-(5,4-d)-pyrimidine (RA 233). Mayo Clin. Proc., 45, 695-699, 1970.

Dixon, J.S. Changes in the fine structure of satellite cells surrounding chromatolytic neurons. Anat. Rec., 163, 101-110, 1969.

Dobell, A.R.C., Wyant, J.D., Seamans, K.B. and Gloor, P. Penicillin epilepsy studies on the blood-brain barrier during cardiopulmonary bypass. J. thorac. cardiovasc.Surg., 52, 469-475, 1966.

Dodrill, F.D., Hill, E. and Gerisch, R. Some physiologic aspects of the artificial heart problem. J. thorac. Surg., 24, 134-150, 1952.

Donald, D.E. and Fellows, J. Relation of temperature, gas tension and hydrostatic pressure to the formation of gas bubbles in extracorporeally oxygenated blood. Surg. Forum, 10, 589-592, 1959.

Dott, N.M. and Gillingham, F.J. Mechanical aspects of the cerebrospinal fluid circulation - physiological, pathological, surgical. In: CIBA Foundation symposium of the cerebrospinal fluid (G.E.W. Wolstenholme and C.M. O'Connor, eds.). London O.U.P., pp246-264, 1958.

Drew, C.E. Profound hypothermia in cardiac surgery. Br. med. Bull., 17, 37-42, 1961.

Drew, C.E. and Anderson, I.M. Profound hypothermia in cardiac surgery. Lancet, vol. I (1969), 748-750.

Drew, C.E., Keen, G. and Benazon, D.B. Profound hypothermia. Lancet, vol. I (1959), 745-747.

Dubost, C., Blondeau, P. and Piwnica, A. Extracorporeal circulation during profound hypothermia with prolonged total circulatory arrest. J. cardiovasc. Surg., 3, 286-293, 1962.

Dunbar, H.S., Guthrie, T.C. and Karpell, B. A study of the cerebrospinal fluid pulse wave. Archs Neurol., Chicago, 14, 624-630, 1966.

Ead, H.W., Green, J.H. and Neil, E. A comparison of the effects of pulsatile and non-pulsatile blood flow through the carotid sinus on the reflexogenic activity of the sinus baroreceptors in the cat. J. Physiol., Lond., 118, 509-519, 1952.

Edmunds, L.H. Prevention of brain damage during profound hypothermia and circulatory arrest with Rheomacrodex. Rheomacrodex Reports of Symposia, vol. 2, 32-34, 1964.

Edmunds, L.H., Folkman, J., Snodgress, A. and Brown, R.B. Prevention of brain damage during profound hypothermia and circulatory arrest. Ann. Surg., 157, 637-649, 1963.

Edstrom, R.F.S. and Essex, H.E. Swelling of the brain induced by anoxia. Neurology, Minneap., 6, 118—124, 1956.

Egerton, N. and Kay, J.H. Psychological disturbances associated with open heart surgery. Br. J. Psychiat., 110, 433-439, 1964.

Ehrenhaft, J. L. and Claman, M.A. Cerebral complications of open-heart surgery. J. thorac. cardiovasc. Surg., 41, 503-508, 1961.

Ehrenhaft, J.L., Claman, M.A., Layton, J.M. and Zimmerman, G.R. Cerebral complications of open-heart surgery: Further observations. J. thorac. cardiovasc. Surg., 42, 514-526, 1961.

Emmons, P.R., Harrison, M.J.G., Honour, A.J. and Mitchell, J.R.A. Effect of a pyrimodopyrimidine derivative on thrombus formation in the rabbit. Nature, Lond., 208, 255-257, 1965.

Enerson, D.M. Cellular swelling: I. Hypothermia graded hypoxia, and the osmotic effects of low molecular weight dextran on isolated tissue. Ann. Surg., 163, 169-174, 1966.

Evans, E.A. and Wellington, J.S. Emboli associated with cardiopulmonary bypass. J. thorac. cardiovasc. Surg., 48, 323-330, 1964.

Filistovich, V.I., Gero, Y.I. and Gerova, M.A. O znachenii amplitudy pul'sovykh kolebanii davleniya dlya aktivnosti baroretseptorov karotidnogo sinusa. Fiziol. Zh. SSSR, IM I M Sechenova 52, 874-882, 1966.

Fischer-Williams, M. and Cooper, R.A. Some aspects of electro-encephalographic changes during open-heart surgery. Neurology, Minneap., 14, 472-482, 1964.

Fishman, A.P. Pulsatile pressures and flows in the pulmonary circulation. In: The lung (A.A. Liebow and D. E. Smith, eds.). Baltimore, Williams and Wilkins, pp151-163, 1968.

Fishman, N.H., Carlsson, E. and Roe, B.B. The importance of the pulmonary veins in systemic air embolism following open-heart surgery. Surgery, St. Louis, 66, 655-662, 1969.

Fiszer, S. and De Robertis, E. Subcellular distribution and chemical nature of the receptor for 5-hydroxytryptamine in the central nervous system. J. Neurochem., 16, 1201-1209, 1969.

Fowle, A.S.E. The physiology of shock. Br. J. hosp. med., I 307-311, 1968.

Fowler, W.M., Kavan, E.M. and Walter, R.D. Open-heart surgery. Neurological findings and electroencephalographic patterns before, during, and after open-heart surgery (Two-year follow-up). Am. J. Dis. Child., 104, 131-141, 1962.

Friede, R.L. Interpretation of hyperchromic nerve cells. Relative significance of the type of fixative used, and of the osmolarity of the cytoplasm and the surrounding fluid in the production of cell shrinkage. J. comp. Neurol., 121, 137-149, 1963.

Galletti, P.M. and Brecher, G.A. Heart-lung bypass: Principles and techniques of extracorporeal circulation. New York, Grune and Stratton, pp 207-212, 1962.

Galletti, P.M. and Brecher, G.A. Artificial blood circulation. In: Handbook of physiology, section 2 Circulation, vol. 3 (W.F. Hamilton and P. Dow, eds.). Washington, American Physiological Society, pp2123-2161, 1965.

Gesell, R.A. On the relation of pulse pressure to renal secretion. Am. J. Physiol., 32, 70-93, 1913.

Gibbon, J.H. Artificial maintenance of circulation during experimental occlusion of pulmonary artery. Archs Surg., London., 34, 1105-1131, 1937.

Gilberstadt, H. and Sako, Y. Intellectual and personality changes following open-heart surgery. Archs gen. Psychiat., 16, 210-214, 1967.

Gilman, S. Cerebral disorders after open-heart operations. New Engl. J. Med., 272, 489-498, 1965.

Giron, F., Birtwell, W.C., Soroff, H.S. and Deterling, R.A. Hemodynamic effects of pulsatile and nonpulsatile flow. Archs Surg., Chicago, 93, 802-810, 1966.

Goldman, A., Boszormanyi, E., Utsu, F., Enescu, V., Swan, H.J.C. and Corday, E. Veno-arterial pulsatile partial bypass for circulatory assist. Dis. Chest, 50, 633-640, 1966.

Goodyer, A.V.N. and Glenn, W.W.L. Relation of arterial pulse pressure to renal function. Am. J. Physiol., 1967, 689-697, 1951.

Gray, E.G. and Guillery, R.W. A note on the dendritic spine apparatus. J. Anat., 97, 389-392, 1963.

Greenfield, J.C. and Tindall, G.T. Effect of acute increase in intracranial pressure on blood flow in the internal carotid artery of man. J. clin. Invest., 44, 1343-1351, 1965.

Greenfield, J.G. and Meyer, A. General pathology of the nerve cell and neuroglia. In: Greenfield's neuropathology, 2nd edit. (W. Blackwood, W.H.McMenemey, A. Meyer and R.M. Norman, eds.). London, Arnold, pp1-70, 1963.

Guillery, R.W. Some electron microscopical observations of degenerative changes in central nervous synapses. In: Progress in brain research, vol. 14 (M. Singer and J. P. Schadé, eds.). Amsterdam, Elsevier, pp57-76, 1965.

Guthrie, T.C., Dunbar, H.S. and Morosini, C.J. A study of the cerebrospinal fluid pulse wave. Trans. Am. neurol. Ass., 91, 244-245, 1966.

Hager, H. Electron microscopical observations on the early changes in neurons caused by hypoxidosis and on the ultrastructural aspects of neuronal necrosis in the cerebral cortex of mammals. In: Selective vulnerability of the brain in hypoxaemia (J.P. Schadé and W.H.McMenemey, eds.). Oxford, Blackwell, pp125-136, 1963.

Hamit, H.F., Beall, A.C. and DeBakey, M.E. Hemodynamic influences upon brain and cerebrospinal fluid pulsations and pressures. J. trauma, 5, 174-183, 1965.

Harden, A. Effects of arterial and venous pressure changes on the electroencephalogram during cardiac operations. Br. med. J. 5426, 23-27, 1965.

Harper, A.M. The inter-relationship between a P_{CO2} and blood pressure in the regulation of blood flow through the cerebral cortex. Acta Neurol. scand., suppl. 14, 94-103, 1965.

Hazán, S.J. Psychiatric complications following cardiac surgery. Part I. A review article. J. thorac. cardiovasc. Surg., 51, 307-319, 1966a.

Hazán, S.J. Psychiatric complications following cardiac surgery. Part II. A working hypothesis - the chemical approach. J. thorac. cardiovasc. Surg., 51, 320-325, 1966b.

Hill, J.D., Aguilar, M.J., Baranco, A., de Lanerolle, P. and Gerbode, F. Neuropathological manifestations of cardiac surgery. Ann. thorac. Surg., 7, 409-419, 1969.

Hinglais, J.R., D'Oléon, F., Chevrier, J-L. and Dubost, C. A time and volume controlled extracorporeal pump. Technical description, performance and experimental application to left heart support. Trans. Am. Soc. artif. internal Organs, 13, 317-321, 1967.

Hirsch, H., Swank, R.L., Breuer, M. and Hissen, W. Screen filtration pressure of homologous and heterologous blood and electroencephalogram. Am. J. Physiol., 206, 811-814, 1964.

Hodges, P.C., Sellers, R.D., Story, J.L., Stanley, P.H., Torres, F. and Lillehei, C.W. The effects of total cardiopulmonary by-pass procedures upon cerebral function evaluated by the electroencephalogram and a blood brain barrier test. A clinical and experimental investigation. In: Extracorporeal circulation (J.G. Allen, ed.). Springfield, Illinois, Thomas, pp279-294, 1958.

Hodgkin, A.L. The ionic basis of electrical activity in nerve and muscle. Biol. Rev., 26, 339-409, 1951.

Hoff, H.F. and Hayes, T.L. Description of a pulsatile-circulatory artery perfusion system. Pflügers Arch. ges. Physiol., 305, 292-294, 1969.

Homburger, E., Himwich, W.A., Etsten, B., York, G., Maresca, R. and Himwich, H.E. Effect of Pentothal anesthesia on canine cerebral cortex. Am. J. Physiol., 147, 343-345, 1946.

Hooker, D.R. A study of the isolated kidney: The influence of pulse pressure upon renal function. Am. J. Physiol., 27, 24-44, 1910.

Horecky, J., Sujansky, E., Cerven, J. and Pappova, E. Some problems of extracorporeal circulation with respect to cerebral circulation. J. cardiovasc. Surg., 7, 79-83, 1966.

Huntington, R.D. The origin of the reactive cells in cerebral stab wounds. J. Neuropath. exp. Neurol., 25, 646-653, 1966.

Ida, Y. Experimental studies on carbohydrate metabolism during heart lung bypass with special reference to a comparison of pulsatile flow with non-pulsatile flow. Arch. Jap. chirurgie, 31, 181-196, 1962.

Ingvar, D.H. The pathophysiology of cerebral anoxia. Acta anaesth. scand., suppl. 29, 47-59, 1968.

Jacobs, L.A., Klopp, E.H., Seamone, W., Topaz, S.R. and Gott, V.L. Improved organ function during cardiac bypass with a roller pump modified to deliver pulsatile flow. J. thorac. cardiovasc. Surg., 58, 703-712, 1969.

Jaén, R., Grossmann-Siegert, V., Ortega, M.A., Bello, A. and Suárez, J. Complications in open heart surgery. Analysis of 350 patients. J. cardiovasc. Surg., 11, 129-136, 1970.

Javid, H., Tufo, H.M., Najafi, H., Dye, W.J., Hunter, J.A. and Julian, O.C. Neurological abnormalities following open-heart surgery. J. thorac. cardiovasc. Surg., 58, 502-509, 1969.

Jenevein, E.P. and Weiss, D.L. Platelet microemboli associated with massive blood transfusion. Am. J. Path., 45, 313-325, 1964.

Jennings, R.B., Kaltenbach, J.P. and Sommers, H.M. Mitochondrial metabolism in ischemic injury. Archs Path., 84, 15-19, 1967.

Johnston, G.I., Bailey, D.D. and Swank, R.L. Screen filtration pressure apparatus: Its use in identifying particulate and aggregated materials in blood and other liquids. Digest 7th Int. Conf. Med. Biol. Eng., Stockholm, p424, 1967.

Johnston, J.B., Ushiro, C., Finley, K.H. and Gerbode, F. Profound hypothermia with prolonged circulatory arrest. An experimental study. Thorax, 21, 391-400, 1966.

Jones, E.G. and Powell, T.P.S. Electron microscopy of the somatic sensory cortex of the cat. I. Cell types and synaptic organization. Phil. Trans. R. Soc., Ser.B 257, 1-11, 1970.

Jordan, P., Tolstedt, G.E. and Beretta, F.F. Microbubble formation in artificial oxygenation. Surgery, St. Louis, 43, 266-269, 1958.

Judson, W.E. and Rausch, N.H. The effects of acute reduction of renal artery blood pressure on renal hemodynamics and excretion of electrolytes and water. J. Lab. clin. Med., 50, 923, 1957.

Karlsson, U. and Schultz, R.L. Fixation of the central nervous system for electron microscopy by aldehyde perfusion. I. Preservation with aldehyde perfusates versus direct perfusion with osmium tetroxide with special reference to membranes and the extracellular space. J. Ultrastruct. Res., 12, 160-186, 1965.

Karlsson, U. and Schultz, R.L. Fixation of central nervous system for electron microscopy by aldehyde perfusion. III. Structural changes after exsanguination and delayed perfusion. J. Ultrastruct. Res., 14, 47-63, 1966.

Keen, G. and Dowlatshahi, K. The effects of circulatory arrest during profound hypothermia upon human myocardial fine structure. Cardiovasc. Res., 4, 348-354, 1970.

Kennedy, J.A. and Bakst, H. The influence of emotions on the outcome of cardiac surgery: A predictive study. Bull. N.Y. Acad. Med., 42, 811-845, 1966.

Khattab, F.I. Alterations in acid phosphatase bodies (lyosomes) in cat motoneurons after asphyxiation of the spinal cord. Expl Neurol., 18, 133-140, 1967.

Koehlstaedt, K.G. and Page, I.H. The liberation of renin by perfusion of kidneys following reduction of pulse pressure. J. exp. Med., 72, 201-216, 1940.

Koenig, H., Groat, R.A. and Windle, W.F. A physiological approach to perfusion-fixation with formalin. Stain Technol., 20, 13-22, 1945.

Koenig, R.S. and Koenig, H. An experimental study of post mortem alterations in neurons of the central nervous system. J. Neuropath. exp. Neurol., 11, 69-78, 1952.

Kornfeld, D.S., Zimberg, S. and Malm, J.R. Psychiatric complications of open-heart surgery. New Engl. J. Med., 273, 287-292, 1965.

Koushanpour, E. and McGee, J.P. Effect of mean pressure on carotid sinus baroreceptor response to pulsatile pressure. Am. J. Physiol., 216, 599-603, 1969.

Kuchibaa, K. Study on prolonged circulatory arrest under profound hypothermia. Effect on cerebral metabolism of circulatory arrest. Acta med., Fukuoka, 36, 602-619, 1966.

Kuwana, K. Experimental and clinical studies on profound hypothermia. Arch. Jap. chirurgie, 31, 158-180, 1962.

Kvitnitskii-Ryzhov., Y.N. and Pelipas, S.I. K voprosy o proiskhozhdenii sklerozirovannykh nervenykh kletok golovnogo mozga. Arkh. Patol., 30, 12-17, 1968.

Laitinen, L. Origin of arterial pulsation of cerebrospinal fluid. Acta Neurol. scand., 44, 168-176, 1968.

Lassen, N.A. Autoregulation of cerebral blood flow. Circulation Res., 15, suppl. I, 201-204, 1964.

Lee, J.C., Glover, M.B., Gilboe, D.D. and Bakay, L. Electron microscopy of isolated dog brain. Expl Neurol., 20, 111-119, 1968.

Lee, W.H., Krumhaar, D., Fonkalsrud, E.W., Schjeide, O.A. and Maloney, J.V. Denaturation of plasma proteins as a cause of morbidity and death after intracardiac operations. Surgery, St. Louis, 50, 29-39, 1961.

Lee, W.H., Miller, W., Rowe, J., Hairston, P. and Brady, M.P. Effects of extracorporeal circulation on personality and cerebration. Ann. thorac. Surg., 7, 562-570, 1969.

Le Gallois, M. Expériences sur le principe de la vie. Paris, D'Hautel, 362pp, 1812.

Lesage, A.M., Freese, J.W., Sealy, W.C. and Young, W.G. Study of the effects of complete circulatory arrest in the profoundly hypothermic dog. Surg., Forum, 11, 188-189, 1960.

Lev., M., Birkner, M.L., Idriss, F.S., Grana, L. and Lark, R.G. Extracorporeal circulation effect on dog's heart. Archs Path., 79, 257-269, 1965.

Lindberg, D.A.B., Lucas, F.V., Sheagren, J. and Malm, J.R. Silicone embolization during clinical and experimental heart surgery employing a bubble oxygenator. Am. J. Path., 39, 129-144, 1961.

Lindbergh, C.A., Perry, V.P., Malinin, T.I. and Mouer, G.H. An apparatus for the pulsating perfusion of whole organs. Cryobiology, 3, 252-260, 1966.

Lindenberg, R. The pathology of the arterial border zones of the brain. J. Neuropath. exp. Neurol., 18, 348-349, 1959.

Lindenberg, R. Patterns of CNS vulnerability in acute hypoxaemia, including anaesthesia accidents. In: Selective vulnerability of the brain in hypoxaemia (J.P. Schade and W.H. McMenemey, eds.). Oxford, Blackwell, pp189-209, 1963.

Litwak, R.S., Slonim, R., Wisoff, G. and Gadboys, H.L. Homologous-blood syndrome during extracorporeal circulation in man. II. Phenomena of sequestration and desequestration. New Engl. J. Med., 268, 1377-1382, 1963.

MacDonald, M. and Spector, R.G. The influence of anoxia on respiratory enzymes in rat brain. Br. J. exp. Path., 44, 11-15, 1963.

Mandelbaum, I., Berry, J., Silbert, M., Burns, W.H. and Rothe, C. Regional blood flow during pulsatile and nonpulsatile perfusion. Archs. Surg., Lond., 91, 771-774, 1965.

Mandelbaum, I. and Burns, W.H. Pulsatile and nonpulsatile blood flow. J. Am. med. Ass., 191, 657-660, 1965.

Many, M., Giron, F., Birtwell, W.C., Deterling, R.A. and Soroff, H.S. Effects of depulsation of renal blood flow upon renal function and renin secretion. Surgery, St. Louis, 66, 242-249, 1969.

Many, M., Soroff, H.S., Birtwell, W.C. and Deterling R.A. The effects of bilateral renal artery depulsation on renin levels. Surg., Forum, 19, 387-389, 1968a.

Many, M., Soroff, H.S., Birtwell, W.C., Giron, F., Wise, H. and Deterling, R.A. The physiologic role of pulsatile and nonpulsatile blood flow. II. Effects on renal function. Archs Surg., 95, 762-767, 1967.

Many M., Soroff, H.S., Birtwell, W.C., Wise, H.M. and Deterling, R.A. The physiologic role of pulsatile and nonpulsatile blood flow. III. Effects of unilateral renal artery depulsation. Archs Surg., 97, 917-923, 1968b.

Maroon, J.C., Edmonds-Seal, J. and Campbell, R.L. An ultrasonic method for detecting air embolism. J. Neurosurg., 31, 196-201, 1969.

Maxwell, D.S. and Kruger, L. The fine structure of astrocytes in the cerebral cortex and their response to injury by heavy ionizing particles. J. cell. Biol., 25, 141-157, 1965.

McCabe, S. Pump for replacement of the heart. Trans. Am. Soc. artif. internal Organs, 5, 289-292, 1959.

McGee-Russell, S.M., Brown, A.W. and Brierley, J.B. A combined light and electron microscope study of early anoxic-ischaemic cell change in rat brain. Brain, 20, 193-200, 1970.

McMaster, P.D. and Parsons, R.J. The effect of the pulse on the spread of substances through tissues. J. exp. Med., 68, 377-400, 1938.

Melrose, D.G., Nahas, R., Alvarez, D., Todd, I.A.D. and Dempster, W.J. Post-operative hypoxia after extracorporeal circulation: A possible graft against host reaction (preliminary communication). Experientia, 21, 47-50, 1965.

Mendler, N. Elektrolyt- und Metabolitveranderungen im Gehirn der Ratte nach Kuhlung auf 4-2°C mit einer Hertzlungenmaschine. Z. ges. exp. Med., 146, 206-225, 1968.

Meyer, B.C., Blacher, R.S. and Brown, F. A clinical study of psychiatric and psychological aspects of mitral surgery. Psychosom. Med., 23, 194-218, 1961.

Millen, J.W. and Woollam, D.H.M. The anatomy of the cerebrospinal fluid. London, O.U.P., 131pp, 1962.

Miller, D.R., Hallaba, M.A.S. and Steegman, A.T. Effect of profound hypothermia with circulatory arrest in dogs. Special reference to changes in cerebrovascular permeability. Ann. Surg., 161, 272-285, 1965.

Miller, J.A., Fonkalsrud, E.W., Latta, H.L. and Maloney, J.V. Fat embolism associated with extracorporeal circulation and blood transfusion. Surgery, St. Louis, 51, 448-451, 1962.

Morgan, D.H. Psychological problems related to cardiac surgery. Address to the Midland Thoracic Society, Birmingham Medical Institute, February 1970.

Mugnaini, E. 'Dark cells' in electron micrographs from the central nervous system of vertebrates. J. Ultrastruct. Res., 12, 235-236, 1965.

Nakayama, K., Tamiya, T., Yamamoto, K., Izumi, T., Akimoto, S., Hashizume, S., Iimori, T., Odaka, M. and Yazawa, C. High amplitude pulsatile pump in extracorporeal circulation with particular reference to hemodynamics. Surgery, St. Louis, 54, 798-809, 1963.

Nemetschek-Gansler, H. and Becker, M. Die Bedeutung der Perfusionfixierung für die Morphologie des Zentralnervensystems. Acta anat., 57, 152-162, 1964.

Nicholson, A.N., Freeland, S.A. and Brierley, J.B. A behavioural and neuropathological study of the sequelae of profound hypoxia. Brain Res., 22, 327-345, 1970.

Nonoyama, A. Hemodynamic studies on extracorporeal circulation with pulsatile and non-pulsatile blood flows. Arch. Jap. chirurgie, 29, 1381-1406, 1960.

O'Connell, J.E.A. The vascular factor in intracranial pressure and the maintenance of the cerebrospinal fluid circulation. Brain, 66, 204-228, 1943.

Ogata, T., Ida, Y., Nonoyama, A., Takeda, J. and Sasaki, H. A comparative study on the effectiveness of pulsatile and non-pulsatile blood flow in extracorporeal circulation. Arch. Jap. chirurgie, 29, 59-66, 1960.

Owens, G., Adams, J.E., Dawson, R.E., Lance, E.M., Sawyers, J.L. and Scott, H.W. Observed central nervous system responses during experimental employment of various pump-oxygenators. Surgery, St. Louis, 44, 240-254, 1958.

Owens, G., Adams, J.E., McElhannon, F.M., and Youngblood, R.W. Experimental alterations of certain colloidal properties of blood during cardiopulmonary bypass. J. Appl. Physiol., 14, 947-948, 1959.

Packer, L. Size and shape transformations correlated with oxidative phosphorylation in mitochondria. I. Swelling-shrinkage mechanisms in intact mitochondria. J. cell Biol., 18, 487-494, 1963.

Paquet, K.J. Experimentelle Untesuchungen zur Funktion der isolierten Niere bei pulsatorischer und Kontinuierlichen perfusion. Brun's Beitrag Klin. Chir., 211, 463-475, 1965.

Paquet, K.J. Hemodynamic studies on normothermic perfusion of the isolated pig kidney with pulsatile and non-pulsatile flows. J. cardiovasc. Surg., 10, 45-53, 1969.

Parsons, R.J. and McMaster, P.D. The effect of the pulse upon the formation and flow of lymph. J. exp. Med., 68, 353-376, 1938.

Patrick, R.T., Kirklin, J.W. and Theye, R.A. The effects of extracorporeal circulation on the brain. In: Extracorporeal circulation (J.G. Allen, ed.). Springfield, Illinois, Thomas, pp272-278, 1958.

Patterson, R.H. and Kessler, J. Microemboli during cardiopulmonary bypass detected by ultrasound. Surgery Gyn. Obstet., 129, 505-510, 1969.

Păușescu, E., Lugojan, R. and Păușescu, M. Cerebral catecholamine and serotonin metabolism in post-hypothermic brain oedema. Brain, 93, 31-36, 1970.

Peirce, E.C. Extracorporeal circulation for open-heart surgery. Springfield, Illinois, Thomas, 117pp, 1969.

Peters, A. and Kaiserman-Abramof, I.R. The small pyramidal neuron of the rat cerebral cortex. The synapses upon dendritic spines. Z. Zellforsch. mikrosk. Anat., 100, 487-506, 1969.

Pevzner, L.Z. Nucleic acids in the neuron-neuroglia unit in various functional states of the nervous system. Macromolecules and the function of the neuron (Z. Lodin and S.P.R. Rose, eds.). Amsterdam, Excerpta Medica Foundation, pp353-364, 1968.

Pontén, U. and Siesjö, B.K. Brain tissue carbon dioxide changes and cerebral blood flow measurements. Acta neurol. scand., 14, 129-134, 1965.

Popovic, P., Horecky, J. and Popovic, V.P. Instrumental responses in rats after hypothermic cardiopulmonary by-pass. Proc. Soc. exp. biol. Med., 126, 225-228, 1967.

Randall, J.E. and Stacy, R.W. Pulsatile and steady pressure-flow relations in the vascular bed of the hind leg of the dog. Am. J. Physiol., 185, 351-354, 1954.

Raventós, J. The action of fluothane - a new volatile anaesthetic. Br. J. Pharmac. Chemother., 11, 394-410, 1956.

Replogle, R., Levy, M., DeWall, R.A. and Lillehei, R.C. Catecholamine and serotonin response to cardiopulmonary bypass. J. thorac. cardiovasc. Surg., 44, 638-648, 1962.

Reynolds, E.S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. cell Biol., 17, 208-212, 1963.

Ritter, E.R. Pressure flow relations in the kidneys. Alleged effects of pulse pressure. Am. J. Physiol., 168, 480-489, 1952.

Rogers, A. and Dunn, P.M. Intestinal perforation, exchange transfusion and P.V.C. Lancet, Vol. 2 (1969), p1246.

Romanova, N.P. and Prozorovskaya, G.P. Morfologicheskie izmeneniya v golovnom mozgu sobak, perenesshikh kernicheskuyu smert', vyzvannuyu mekhanicheskoi asfiksiei. Sudebno-med. Ekspert., 9, 10-14, 1966.

Rouiller, C. Physiological and pathological changes in mitochondrial morphology. Int. Rev. Cytol., 9, 227-292, 1960.

Rumbaugh, C.L., Davis, D.O. and Gilson, J.M. Fate of experimental autologous emboli. Acta radiol., 9, 450-454, 1969.

Sachdev., N.S., Carter, C.C., Swank, R.L. and Blachly, P.H. Relationship between post-cardiotomy delirium, clinical neurological changes, and EEG abnormalities. J. thorac. cardiovasc. Surg., 54, 557-563, 1967.

Schneider, M. Critical blood pressure in the cerebral circulation. In: Selective vulnerability of the brain in hypoxaemia (J.P. Schadé and W.H. McMenemey, eds.). Oxford, Blackwell, pp7-20, 1963.

Schultz, R.L. and Case, N.M. A modified aldehyde perfusion technique for preventing certain artifacts in electron microscopy of the central nervous system. J. Microsc., 92, 69-84, 1970.

Schwartz, S.I., DeWeese, J.A., Niguadula, F.N., Gabel, P.V. and Mahoney, E.B. Tissue oxygen "tension" at various flow rates of extracorporeal circulation. Surg., Forum, 9, 151-154, 1959.

Schweikert, C.H. and Sickinger, K. Pathologisch-histologische Befunde am Humd nach Anwendung eines extrakorporalen Kreislaufes in Verbindung mit tiefer Hypothermie und temporarem Kreislaufstillstand. Thoraxchirurgie, 8, 371-382, 1960.

Selkurt, E.E. Effect of pulse pressure and mean arterial pressure modification on renal hemodynamics and electrolyte and water excretion. Circulation, 4, 541-551, 1951.

Selman, M.W., McAlpine, W.A. and Ratan, R.S. The effectiveness of various heart-lung machines in the elimination of microbubbles from the circulation. J. thorac. cardiovasc. Surg., 53, 613-617, 1967.

Shariff, G.A. Cell counts in the primate cerebral cortex. J. comp. Neurol., 98, 381-400, 1953.

Shepard, R.B. and Kirklin, J.W. Relation of pulsatile flow to oxygen consumption and other variables during cardiopulmonary bypass. J. thorac. cardiovasc. Surg., 58, 694-702, 1969.

Shepard, R.B., Simpson, D.C. and Sharp, J.E. Energy equivalent pressure. Archs. Surg., 93, 730-740, 1966.

Shumacker, H.B. Comments on the distribution of blood flow. Surgery. St. Louis, 47, 1-16, 1960.

Siggaard-Andersen, O. and Engel, K. A new acid-base nomogram. An improved method for the calculation of the relevant blood acid-base data. Scand. J. clin. Lab. Invest., 12, 177-186, 1960.

Siggaard-Andersen, O., Engel, K., Jørgensen, K. and Astrup, P. A micro method for determination of pH, carbon dioxide tension, base excess and standard bicarbonate in capillary blood. Scand. J. clin. Lab. Invest., 12, 172-176, 1960.

Silverstein, A., Jacobson, E., Kreel, J. and Kreigar, H.P. Effects on the brain of extracorporeal circulation in open-heart surgery. Neurology, Minneap., 10, 987-992, 1960.

Silverstein, A. and Kreiger, H.P. Neurologic complications of cardiac surgery. Archs Neurol., Chicago, 3, 601-605, 1960.

Silverstone, J.T., Tannahill, M.M. and Ireland, J.A. Psychiatric aspects of profound hypothermia in open-heart surgery. J. thorac. cardiovasc. Surg., 70, 193-200, 1970.

Sirakova, I., Sirakov, L., Jílek, L. and Rychlík, I. Changes in nucleic acid metabolism in the brain caused by stagnant hypoxia during ontogeny in the rat. Macromolecules and the function of the neuron (Z.Lodin and S.P.R. Rose, eds.). Amsterdam, Excerpta Medica Foundation, pp302-306, 1968.

Smith, W.T. Cerebral lesions due to emboli of silicone anti-foam in dogs subjected to cardiopulmonary bypass. J. Path. Bact., 80, 9-18, 1960.

Soroff, H.S., Many, M., Birtwell, W.C., Giron, F. and Deterling, R.A. Hemodynamic effects of pulsatile and nonpulsatile blood flow. II. Selective depulsation of the aortic arch and brachiocephalic trunk. Archs Surg., 98, 321-325, 1969.

Spencer, F.C., Rossi, N.P., Yu, S-C. and Koepke, J.A. The significance of air embolism during cardiopulmonary bypass. J. thorac. cardiovasc. Surg., 49, 615-634, 1965.

Stead, E.A. Pressures and pulses. Circulation, 31, 481-484, 1965.

Steegman, A.T. and Miller, D.R. Some histopathologic aspects of cerebral injury in dogs subjected to profound hypothermia and circulatory arrest. Neurology, Minneap., 15, 802-814, 1965.

Sulkin, N.M. Histochemical studies on mucoproteins in nerve cells of the dog. J. biophys. biochem. Cytol., I, 459-468, 1955.

Super, N.A., Gol'dina, B.G., Gutkin, V.S. and Mushegyan, S.A. K genezu subednokardial'nykh krovoizliyanii pri iskusstvennom krovoobrashchenii. Grud. Khir., 9, 42-46, 1967.

Swank, R.L. Alteration of blood on storage: Measurement of adhesiveness of "aging" platelets and leukocytes and their removal by filtration. New Engl. J. Med., 265, 728-733, 1961.

Swank, R.L. and Edwards, M.J. Microvascular occlusion by platelet emboli after transfusion and shock. Microvasc. Res., I, 15-22, 1968.

Swank, R.L., Hirsch, H., Breuer, M. and Hissen, W. Effect of glass wool filtration on blood during extracorporeal circulation. Surgery Gyn. Obstet., 117, 547-552, 1963.

Swank, R.L. and Porter, G.A. Disappearance of micro-emboli transfused into patients during cardiopulmonary bypass. Transfusion, 3, 192-197, 1963.

Symbas, P.N., Abbott, O.A. and Leonard, J. The effects of artificial ventilation on cerebrospinal fluid pressure. J. thorac. cardiovasc. Surg., 54, 126-131, 1967.

Takeda, J. Experimental study of peripheral circulation during extracorporeal circulation, with a special reference to a comparison of pulsatile flow with non-pulsatile flow. Arch. Jap. chirurgie, 29, 1407-1430, 1960.

Thakar, D.S. and Tewari, H.B. Histochemical studies on the distribution of alkaline and acid phosphatases amongst the neurons of the cerebellum, spinal and trigeminal ganglia of bat. Acta histochem., 28, 359-367, 1967.

Theye, R.A. and Michenfelder, J.D. The effect of halothane on canine cerebral metabolism. Anesthesiology, 29, 1113-1118, 1968a.

Theye, R.A. and Michenfelder, J.D. The effect of nitrous oxide on canine cerebral metabolism. Anesthesiology, 29, 1119-1124, 1968b.

Thomassen, R.W., Howbert, J.P., Winn, D.F. and Thompson, S.W. The occurrence and characterization of emboli associated with the use of a silicone antifoaming agent. J. thorac., cardiovasc. Surg., 41, 611-622, 1961.

Trinkle, J.K., Helton, N.E., Wood, R.E. and Bryany, L.R. Metabolic comparison of a new pulsatile pump and a roller pump for cardiopulmonary bypass. J. thorac. cardiovasc. Surg., 58, 562-569, 1969.

Trump, B.F., Smuckler, E.A. and Benditt, E.P. A method for staining epoxy sections for light microscopy. J. Ultrastruct. Res., 5, 343-348, 1961.

Tsuji, H.K., Redington, J.V., Mendez, A. and Kay, J.H. The prevention of air embolism during intracardiac surgery. J. thorac. cardiovasc. Surg., 59, 484-488, 1970.

Van Breeman, V.L. and Bowman, R.W. Lamellar bodies in Purkinje cells of dog cerebellum. J. cellBiol., 31, 138A, 1966.

Van Harreveld, A. Changes in volume of cortical neuronal elements during asphyxiation. Am. J. Physiol., 191, 233-242, 1957.

Van Harreveld, A. Asphyxial changes in the cerebellar cortex. J. cell. comp. Physiol., 57, 101-110, 1961.

Van Harreveld, A. and Khattab., F.I. Electron microscopy of asphyxiated spinal cords of cats. J. Neuropath. exp. Neurol., 26, 521-536, 1967.

Van Harreveld, A. and Schadé, J.P. Chloride movements in cerebral cortex after circulatory arrest and during spreading depression. J. cell. comp. Physiol., 54, 65-77, 1959.

Van Nimwegen, D. and Sheldon, H. Early postmortem changes in cerebellar neurons of the rat. J. Ultrastruct. Res., 14, 36-46, 1966.

Vaynrib, E.A., Frid., E.A., Martinov, L.N., Ananev, M.G., Mushchegyan, S.A. and Levitskaya, L.A. Apparatus for artificial blood circulation. Medskaya Prom, SSSR, II, 50-55, 1957.

Watson, M.,. Staining of tissue sections for electron microscopy with heavy metals. J. biophys. biochem. Cytol., 4, 475-478, 1958.

Webster, H. de F. and Ames, A. Reversible and irreversible changes in the fine structure of nervous tissue during oxygen and gluoose deprivation. J. cell Biol., 26, 885-909, 1965.

Weiss, L. The dynamic nature of the cell periphery. In: The cell periphery, metastasis and other contact phenomena. North-Holland research monographs, Frontiers of Biology, vol. 7 (A. Neuberger and E. Tatum, eds.). Amsterdam, North-Holland Publishing Co., pp75-116, 1967.

Welbourn, N., Melrose, D.G. and Moss, D.W. Changes in serum enzyme levels accompanying cardiac surgery with extracorporeal circulation. J. clin. Path., 19, 220-232, 1966.

Wemple, R.R., Mockros, L.F. and Lewis, F.J. Pulmonary function during pulsatile and nonpulsatile right heart bypass. J. thorac. cardiovasc. Surg., 57, 190-202, 1969.

Wesolowski, S.A. Extracorporeal circulation: Continuous, controlled variation of the frequency, volume and systolic rise time of the pulse. J. Appl. Physiol., 6, 809-814, 1954.

Wesolowski, S.A., Fisher, J.H. and Welch, C.S. Heart-lung by-pass using pumps and isolated homologous lungs. Surgery Gyn. Obstet. 95, 762-771, 1952.

Wesolowski, S.A., Fisher, J.H. and Welch, C.S. Perfusion of the pulmonary circulation by nonpulsatile flow. Surgery, St. Louis, 33, 370-375, 1953.

Wesolowski, S.A., Sauvage, L.R. and Pinc., R.D. Extracorporeal circulation: The role of the pulse in maintenance of the systemic circulation during heart-lung bypass. Surgery, St. Louis, 37, 663-682, 1955.

Westrum, L.E. and Lund, R.D. Formalin perfusion for correlative light- and electron-microscopical studies of the nervous system. J. cell Sci., I, 229-238, 1966.

Wilkens, H., Regelson, W. and Hoffmeister, F.S. The physiologic importance of pulsatile blood flow. New Engl. J. Med., 267, 443-446, 1962.

Williams, B.N. Cerebral injury following cardiac operations. Lancet, vol. I (1964), 221-222.

Willman, V.L., Zafiracopoulos, P. and Hanlon, C.R. Air embolism. In: Extracorporeal circulation (J.G. Allen, ed.). Springfield, Illinois, Thomas, pp295-302, 1958.

Wollman, H., Stephen, G.W., Clement, A.J. and Danielson, G.K. Cerebral blood flow in man during extracorporeal circulation. J. thorac. cardiovasc. Surg., 52, 558-564, 1966.

Wright, E.S., Sarkozy, E., Dobell, A.R.C. and Murphy, D.R. Fat globulemia in extracorporeal circulation. Surgery, St. Louis, 53, 500-504, 1963.

Wright, E.S., Sarkozy, E., Harpur, E.R., Dobell, A.R.C. and Murphy, D.R. Plasma protein denaturation in extracorporeal circulation. J. thorac. cardiovasc. Surg., 44, 550-556, 1962.

Wright, G. and Sanderson, J.M. Improved method for fixation of dog brain by vascular perfusion. J. Path., 100, 295-305, 1970.

Yap, S-L. and Spector, R.G. Intracellular enzyme changes in post-anoxic rat brain. Br. J. exp. Path., 46, 422-432, 1965.

Zingg, W. and Kantor, S. Observations on the temperatures in the brain during extracorporeal differential hypothermia. Surg., Forum, 11, 192-193, 1960.

Zwetnow, N. Cerebral blood flow autoregulation to blood pressure and intracranial pressure variations. Scand. J. clin. Lab. Invest., 22 suppl. 102, VA, 1968.